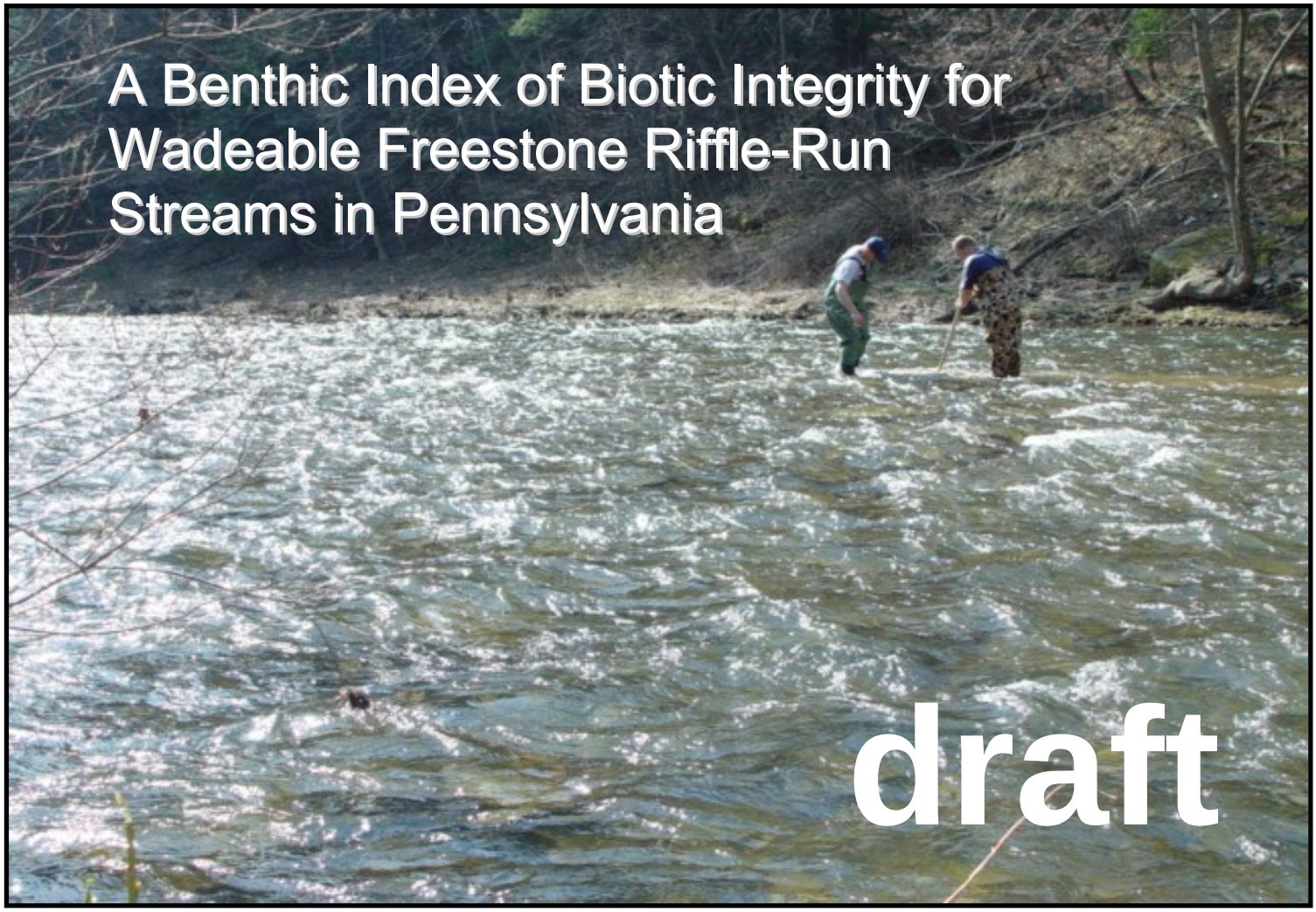
A Benthic Index of Biotic Integrity for Wadeable Freestone Riffle-Run Streams in Pennsylvania



draft

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DEPARTMENT OF ENVIRONMENTAL PROTECTION

Division of Water Quality Assessment and Standards

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The main text of this report consists of about 50 pages, with the remaining 60-some pages comprised of supporting appendices. Many of the figures in the text and appendices depend on color for full comprehension and are best viewed in electronic format. In an effort to help conserve resources, please print only as much of this report as you deem critically necessary.

THANK YOU!

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The participants in the Pennsylvania Tiered Aquatic Life Use workshops, who also provided invaluable input and guidance for this project, are listed in the table immediately below.

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	2006	2007	2008			
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Charlie McGarrell	X					
Steve Moyer			X			
Jen Orr		X	X			
Molly Pulket		X	X			
Rob Ryder	X	X	X			
Tony Shaw		X				
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INTRODUCTION

This project aims to develop a scientifically credible indicator of biological integrity for benthic macroinvertebrate communities in Pennsylvania's wadeable freestone streams. Such an indicator will assist in guiding and evaluating legislation, policy and management strategies as well as setting goals for aquatic resources by enabling direct quantification of important ecological attributes along a gradient of biological conditions and ecosystem stressors (Davis and Simon 1995; Davies and Jackson 2006; Hawkins 2006). This indicator can serve as a measure of the extent to which anthropogenic stressors impair the capability of a stream to support a healthy aquatic community (Davis and Simon 1995).

Legislative Background

The objective of the United States Federal Water Pollution Control Act (United States Code 2006: Title 33, Sections 1251 through 1387) – more commonly known as the Clean Water Act (CWA) – as stated in section 1251(a) "is to restore and maintain the chemical, physical, and biological integrity of the Nation's waters." An interim goal of the CWA as stated in Section 1251(a)(2) is "… water quality which provides for the protection and propagation of fish, shellfish, and wildlife…" Section 1251(b) of the CWA indicates that the primary authority and responsibility for prevention, reduction and elimination of pollution as well as for management of land and water resources rests with the States. Thus, States are responsible for setting water quality goals to protect aquatic life. To this end, States have defined various levels of designated aquatic life use (ALU) to be protected for specific water bodies (e.g., recreational fishing, fish migration).

The Pennsylvania Code (2008: Title 25, Chapter 93.3) recognizes four categories of protected ALUs, including: (1) cold water fishes (CWF); (2) warm water fishes (WWF); (3) migratory fishes (MF); and (4) trout stocking (TSF). The CWF and WWF uses include protection of fish as well as additional flora and fauna (e.g., benthic macroinvertebrates) indigenous to a cold or warm water habitat, respectively. The TSF use also included protection of fish and additional flora/fauna indigenous to a warm water habitat. Pennsylvania recognizes two antidegradation – or "special protection" – water uses: high quality waters (HQ) and exceptional value waters (EV). Details concerning these uses and their application to Pennsylvania's waters can be found in Chapter 93 of the Pennsylvania Code.

Biological Monitoring

To meet the objectives outlined in the CWA, evaluations of aquatic ecosystem integrity ideally include a physical habitat evaluation (e.g., flow regimes, types and distribution of substrate), an evaluation of water chemistry (e.g., concentrations of toxic and non-toxic chemicals, temperature measurements) and an evaluation of biological communities (e.g., fish, benthic macroinvertebrates, periphyton). However, chemical water quality evaluations are of limited value in assessing overall ecosystem condition for a number

of reasons, including: difficulty evaluating every relevant chemical parameter and synergistic chemical effects on ecosystems; the highly transient nature of lotic water chemistry; as well as the expense and logistical considerations of continuous chemical water quality monitoring, or even monitoring that is frequent enough to adequately characterize chemical conditions at a site for all relevant parameters. Physical habitat evaluations, though informative in many respects, are also of limited value in assessing overall ecosystem integrity for a wide array of stressors. For example, in cases of acid deposition in an otherwise "pristine" area, physical habitat conditions may be excellent (e.g., wide, forested riparian zone, diversity of velocity/depth regimes), but the biological community may be severely impacted from atmospheric acid stress.

Biological monitoring offers the ability to assess long-term, cumulative effects of many types of ecosystem stress, including stress related to chemical and physical habitat factors. Organisms living in aquatic environments are intimately associated with and affected by chemical water quality and physical habitat conditions. As such, these organisms can be viewed as living indicators of overall ecosystem condition. However, biological monitoring also has its limitations and cannot always identify causative stressors, which may be better identified when biological data is viewed in conjunction with information from chemical and physical habitat assessments (Novotny 2004).

There is some debate as to which one of the three CWA categories of integrity is most meaningful. The United States Environmental Protection Agency (USEPA) requires that if any one category of an aquatic ecosystem assessment indicates impairment of a use for a water body, then the overall integrity is impaired (USEPA 1994). Others contend that biological assessments should be given the highest priority given that biological assemblages often reflect cumulative effects of stresses in an ecosystem (see Novotny 2004). Regardless of this debate, indices of biological integrity based on direct measures of community and population response provide relevant and useful tools that can be used independently, or in concert with other information for the purpose of assessing ALUs (Novotny 2004).

Indicators of Biological Integrity

Although the CWA outlines the general objective of biological integrity, no legislation explicitly defines biological integrity. The United States House and Senate Committee on Public Works deliberations on the CWA included the concept of "naturalness" as a key part of biological integrity (see Stoddard et al. 2006). Legislation in the United States, Europe and Australia expresses a need to characterize biological conditions that would occur in a natural state, free from human impacts (Stoddard et al. 2006).

Consistent with this concept, a definition of biological integrity proposed by some ecologists states that an ecosystem with biological integrity supports and maintains a balanced, integrated, adaptive system having a full range of ecosystem elements (e.g., genes, species, assemblages) and processes (e.g., mutation, metapopulation dynamics, nutrient and energy dynamics) expected in areas with no or minimal human influence (Karr and Dudley 1981; Davis and Simon 1995; Davies and Jackson 2006).

Monitoring and assessment of the biological integrity of inland water resources across the world frequently involves measuring the degree to which community-level biological attributes (e.g., structure, composition, function, diversity) differ from a reference community (Davis and Simon 1995; Davies and Jackson 2006; Hawkins 2006; Stoddard et al. 2006). Generally, the major goal of biological monitoring and assessment is to describe the impacts of human activities on the structure and function of aquatic ecosystems (Stoddard et al. 2006).

Accurate assessment of biological condition requires integration of biological responses at varying scales, from individual organism responses to community-level responses to ecosystem-level responses (Barbour et al. 1995). Past efforts have helped develop and refine the science of using biological indicators to assess ecosystem conditions (Hawkins 2006). Such indicators of biological integrity help to document environmental conditions at community and ecosystem levels, which can assist in diagnostic analyses of sources and causes of ecosystem stress (Barbour et al. 1995).

Numerous other States have developed and are using indices of biological integrity based on stream benthic macroinvertebrate communities as ALU assessment tools, including Maryland (Stribling et al. 1998); West Virginia (Gerritsen et al. 2000); Virginia (Burton and Gerritsen 2003); and Kentucky (Pond et al. 2003), among many others.

Pennsylvania's streams

The Commonwealth of Pennsylvania encompasses approximately 45,000 square miles of land (Figure 1) with diverse climatic, geological, physiographic and land use characteristics. Well over 80,000 miles of streams drain Pennsylvania's varied landscape, ranging from ephemeral, headwater creeks and streams to great rivers such as the Ohio, Delaware and Susquehanna. The Pennsylvania Department of Environmental Protection (DEP) recognizes that certain types of streams naturally differ in physiochemical characteristics and, subsequently, in biological potential. For example, benthic macroinvertebrate communities in true limestone spring streams – those streams heavily influenced by springs and groundwater flow in areas of primarily calcareous geology – exhibit noticeably different characteristics than communities in freestone streams; these differences are attributable in large part to the unique physiochemical conditions associated with spring-fed, groundwater-dominated streams (e.g., relatively constant temperature and flow regimes). Currently, DEP uses separate methodologies to monitor and assess benthic macroinvertebrate communities in lower gradient pool-glide type streams, true limestone spring streams and wadeable freestone riffle-run type streams, the last of these stream types being the focus of this project.

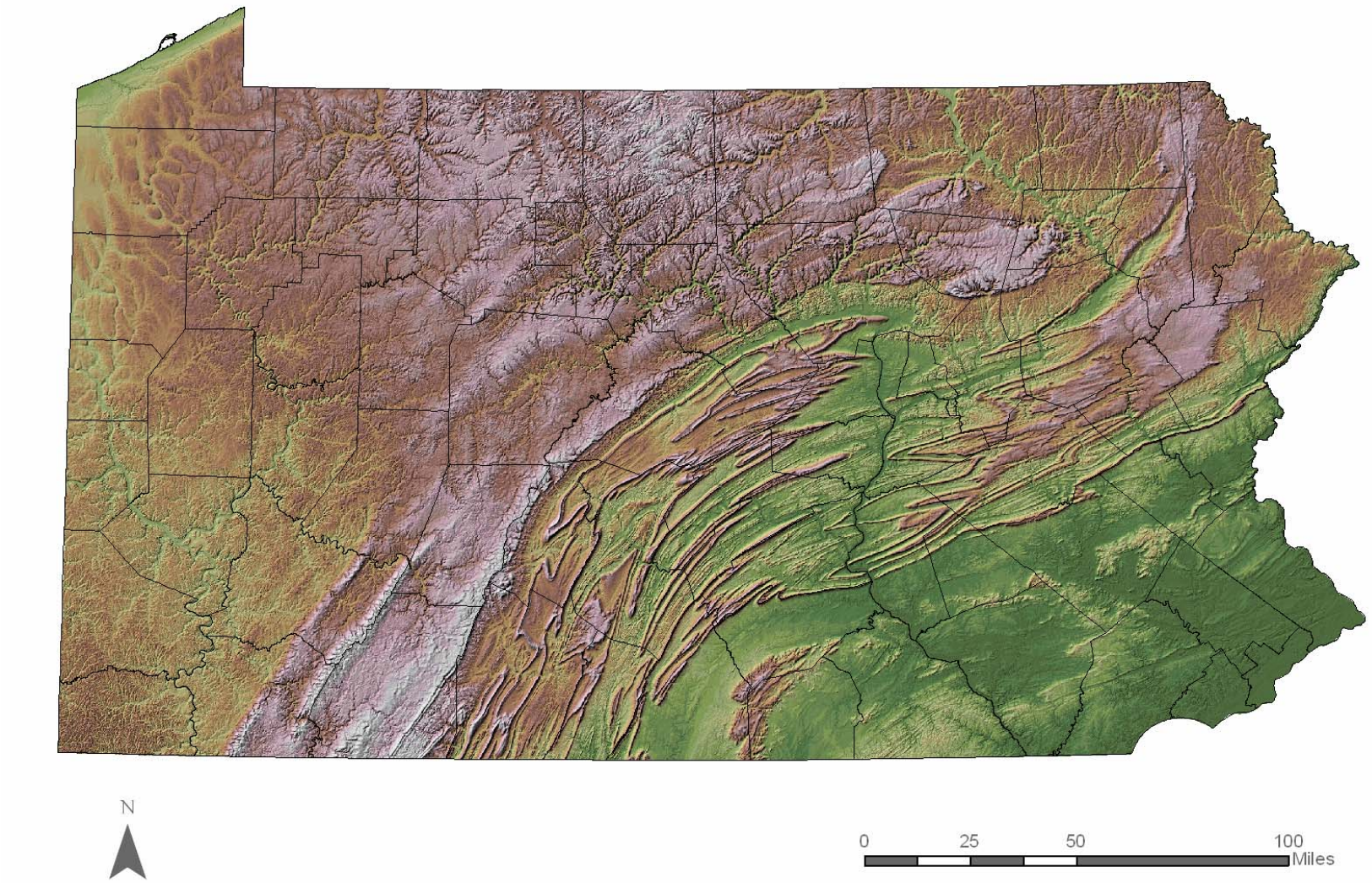

Figure 1. Shaded relief map of Pennsylvania (with county boundaries).

DATA COLLECTION METHODS

All benthic macroinvertebrate samples analyzed in this project were collected using D-frame nets with 500-micron mesh. Field sampling and laboratory methods are more fully described in Appendix A. Working progressively upstream, biologists composited six kicks from riffle areas distributed throughout a 100-meter stream reach, with each kick disturbing approximately one square meter immediately upstream of the net for approximately one minute to an approximate depth of 10 cm, as substrate allowed. Composited samples were preserved with 95% ethanol in the field and transported back to the laboratory for processing. In the lab, each composited sample was placed into a 3.5" deep rectangular pan (measuring 14" long x 8" wide on the bottom of the pan) marked off into 28 four-square inch (2" x 2") grids. Four of the grids were randomly selected, their contents were extracted using a four-square inch circular "cookie cutter," and placed into another identical empty pan. All the organisms were picked from this second pan. If less than 160 identifiable organisms were picked from the second pan, additional grids were randomly selected and extracted from the first pan, transferred to the second pan and picked until the target number of organisms (200 $\pm$ 40 organisms) was obtained. If more than 240 identifiable organisms were picked from the original four grids then the second pan was cleared of debris, the picked organisms were floated in the cleared pan and randomly-selected grids were picked until the target number of organisms was obtained. Any grids selected during this entire process were picked in their entirety and the total numbers of grids selected for each part of the sub-sampling process were recorded.

Organisms in the sub-sample were identified and counted. Midges were identified to the family level of Chironomidae. Snails, clams and mussels were all also identified to family levels. Roundworms and proboscis worms were identified to the phylum levels of Nematoda and Nemertea, respectively. Moss animacules were identified to the phylum level of Bryozoa. Flatworms and leeches were identified to the class levels of Turbellaria and Hirudenia, respectively. Segmented worms, aquatic earthworms, and tubificids were identified to the class level of Oligochaeta. All water mites were identified as Hydracarina, an artificial taxonomic grouping of several mite superfamilies. All other macroinvertebrates were identified to genus level.

Land use values were calculated for the drainage area upstream of each sampling location using ESRI ® ArcMap™ 9.2 software and a statewide Enhanced Thematic Mapper satellite land cover dataset produced by Penn State University in 2001. Biologists collected water chemistry samples and conducted physical habitat assessments concurrently with many macroinvertebrate samples, although not all macroinvertebrate samples in the dataset had accompanying water chemistry and habitat data.

In addition to benthic macroinvertebrates, land use, water chemistry and physical habitat data, a suite of GIS-based data were included in the analysis for each sample, including: watershed area; geographic information (e.g., river basin, hydrologic unit code, ecoregion, physiographic province); sampling location elevation; designated use

and current ALU attainment status of the stream segment from which the sample was taken; geologic composition of the watershed; slope and slope class (low gradient ≤ 0.5% slope; moderate gradient = 0.51% to 2% slope; high gradient > 2%) of the stream segment from which the sample was taken – as determined by The Nature Conservancy (Anderson and Olivero 2003); stream order from the 1:100,000 scale National Hydrography Dataset (NHD) Plus (http://www.horizon-systems.com/nhdplus/); and longitude and latitude coordinates of the sampling location.

Numerous biologists collected the data used in this analysis from July 1999 to August 2008. The samples in the dataset were collected for a variety of DEP survey types, with most samples collected as part of in-stream comprehensive evaluations and antidegradation surveys. Some samples in this dataset were also collected as probabilistic surveys, long-term fixed-site water quality network monitoring surveys, intensive unassessed follow-up surveys, effluent dominated surveys, cause effect surveys and use attainability surveys.

In areas with multiple samples taken within a short distance (i.e., within a few hundred meters on the same stream reach) on different visits, the most recent sample was used in analyses to avoid spatial overrepresentation of more intensively sampled stream reaches, unless there was reason to suspect a major difference between spatially proximate samples (e.g., samples upstream and downstream of a discharge), in which case all samples were retained in analyses because they represent different conditions.

DEFINITION OF THE STRESSOR GRADIENT

Conceptual Background and Literature Review

A critical step in development and implementation of any indicator of biological integrity used to evaluate effects of human activities on stream ecosystems involves quantification and comparison of the current condition of a stream's biology to a standard or benchmark condition. The standard or benchmark condition is often referred to as the reference condition and can be defined for a given type of water body and a given ALU (Hughes 1995; Barbour et al. 1999; Hawkins 2006; Stoddard et al. 2006). This reference condition represents the desired state of biotic assemblages based on undisturbed conditions representative of a region and serves as the foundation for development of biological criteria (Hughes 1995; Stoddard et al. 2006). Reference conditions must be tailored to certain regions or certain types of water bodies because attainable biological conditions cannot be expected to be the same for every region or type of water body. For example, one would expect naturally different biological conditions in a stream in a tropical rainforest than in an arctic lake. The reference condition is usually defined as a range of conditions resulting from natural temporal and spatial variation and sampling error (Hughes 1995; Stoddard et al. 2006). Definition of the reference condition can be revisited and refined as more samples are collected and analyzed from more sites (Hughes 1995).

Expectations of biological condition can be estimated in a number of ways, including: the reference site approach (i.e., comparison to minimally or least disturbed sites); best professional judgment; interpretation of historical conditions; extrapolation of empirical models; and evaluation of ambient distributions (Hughes 1995; Stoddard et al. 2006). Each method of determining the reference condition has its own strengths and weaknesses and each method relies on ecosystem classification to some degree (Hughes 1995). The most useful means of defining the reference condition draw on all of these approaches (Hughes 1995).

Although the process of defining the reference condition should be as objective as possible (e.g., use of defined abiotic criteria), considerable professional judgment is involved in site selection, data analysis and subsequent determination of acceptable versus unacceptable indicator scores (Hughes 1995). Best professional judgment can be difficult to quantify, but it plays an important role in any method of defining the reference condition (Hughes 1995) and can be strengthened when used in concert with other methods, such as abiotic criteria. Experienced biologists can develop empirical understanding of biological conditions in the absence of substantial human disturbance (Stoddard et al. 2006). The scientific credibility of best professional judgment improves if it is tied to sound ecological theory, can be replicated by similarly experienced peers, and any "decision rules" can be documented or quantified (Stoddard et al. 2006). The discussion later in this paper about DEP's tiered aquatic life use (TALU) workshops further explores the scientific credibility of applying best professional judgment to macroinvertebrate communities in Pennsylvania's wadeable freestone streams.

Stoddard et al. (2006) argue that the term “reference condition” should be used consistently to refer to a state of “naturalness” of the biotic structure and function, and that “naturalness implies the absence of significant human disturbance or alteration.” Stoddard et al. (2006) also propose that this “reference condition” should be properly referred to as the “reference condition of biological integrity.” Stoddard et al. (2006) go on to define four additional terms to describe the expected condition to which current conditions are compared, including: (1) “minimally disturbed condition”; (2) “historical condition”; (3) “least disturbed condition”; and (4) “best attainable condition.” Hughes et al. (1998) suggest that the appropriate historical condition for North America includes the impact of indigenous peoples, but excludes the impacts of European immigrants.

In many areas, if not all over the planet, it is difficult to locate sampling sites representative of the natural state, or reference condition of biological integrity, and the goal of “pristine” waters (i.e., free from all human impacts) is an unrealistic goal due to widespread human impacts. As a result, reference conditions and water resource goals often practically describe minimally disturbed, least disturbed or best attainable conditions (Hughes 1995; Novotny 2004; Stoddard et al. 2006). However, it is important to select reference sites representative of a region and ecosystem type that are disturbed as little as possible because the definition of the reference site has important consequences for development of biological indicators and subsequent establishment of ALU attainment thresholds (Hughes 1995; Barbour et al. 1999). For natural resource management purposes, defining the reference condition helps establish the ecological potential of aquatic ecosystem types in a region while taking into account irreversible and reversible changes caused by humans (Novotny 2004). Reference sites representing least-disturbed conditions are moving targets of which human activities and natural processes are a part (Hughes 1995; Stoddard et al. 2006), but the range of conditions defined by what Stoddard et al. (2006) describe as the minimally disturbed condition should serve as a nearly invariant anchor by which to judge current conditions.

For DEP, this project represents a departure from past methods of defining reference conditions. Historically, for antidegradation surveys, DEP used site-specific reference conditions (i.e., comparing a sample taken at one site of undetermined biological condition to one or two other sites of known, reference-quality biological condition) to assess the biological condition of streams. This project defines a reference condition based on a population of sites exhibiting biological integrity from across Pennsylvania to which sites of unknown biological integrity can be compared (Hughes 1995). One major benefit of this population-based reference condition approach is that once the population of sites representing the reference condition is defined, there will no longer be a need for paired sampling of sites for antidegradation surveys as was previously used for the site-specific reference condition approach. In other words, biologists will no longer have to sample two or three sites every time they wish to make a determination of relative biological condition; they will be able to simply sample one site and compare it to the population-based reference condition established in this project. This approach provides comparability of samples for sites across the state from similar types of water bodies (i.e., wadeable freestone streams) and allows more efficient use of limited public resources for monitoring and assessment of aquatic resources over the entire state.

The index of biological integrity developed in this project is not limited in application only to antidegradation surveys; it can also be applied to many other types of surveys, such as cause-effect surveys.

Quantifying the Stressor Gradient

Limited resources, time and data often hinder our ability to holistically assess exposure of stream ecosystems to the full range of stressors that impact them, so suites of criteria are often used to describe the characteristics of sites in a region that are least and most exposed to stressors, representing reference and stressed conditions respectively (Stoddard et al. 2006).

For this project, two abiotic indicators – a land use index and a physical habitat index – were used to assign each sample site to an initial condition tier. The land use and physical habitat indices were calculated as follows:

Land use index = (% forest + % wetland)

Physical habitat index = (Total physical habitat score / 240) * 100

The land use index can range from zero (i.e., a watershed with no forest or wetlands and all urban, agricultural, transitional, quarry and/or mining land use) to 100 (i.e., a watershed covered completely by forest and/or wetland). The physical habitat index can range from five (i.e., a stream segment rated as being in the poorest possible condition – a score of one – in each of the 12 physical habitat evaluation categories) to 100 (i.e., a stream segment rated as being in optimal condition – a score of 20 – in each of the 12 physical habitat categories). The land use and physical habitat indices were used to assign sample sites to one of four initial condition tiers (Table 1).

Table 1. Land use index and physical habitat index values used to assign sample sites to initial condition tiers.

Index	Index value range	Initial condition tier	Number of sites In initial condition tier
Land use index	100 to 80	1	418
	79.9 to 70	2	110
	69.9 to 55	3	211
	54.9 to 0	4	536
Physical habitat index*	100 to 80	1	470
	79.9 to 75	2	178
	74.9 to 65	3	251
	64.9 to 0	4	196

* 1,095 of the 1,275 sites had physical habitat data available

Land use data was available for all sites, but physical habitat data was not available for 162 of the 1,275 sites. The initial condition tiers for each site were assigned by

averaging the condition tiers for the land use index and habitat index, where available. Initial condition tiers defined by land use and physical habitat evaluations were then adjusted based on water chemistry and the presence of other anthropogenic stressors. Where available, water chemistry data was used to adjust initial condition tier assignments to account for impacts not picked up by land use and habitat evaluations (e.g., mine drainage, acid precipitation). Screening values for water chemistry aimed to pick out extreme values for eleven parameters (Table 2).

Table 2. Water chemistry screening values and data availability.

Parameter	Screening value	Number of samples with data	Number of samples above/below screening value
pH – field	pH ≤ 5.5 with Alkalinity ≤ 5	727	37
pH – lab		159	
Alkalinity – field (mg/L as $CaCO_3$)		567	
Alkalinity – lab (mg/L as $CaCO_3$)		167	
Specific Conductivity – field (μmho/cm)	≥ 500	707	46
Specific Conductivity – lab (μmho/cm)		58	
Total Iron – lab (μg/L)	≥ 1,000	95	12
Total Manganese – lab (μg/L)	≥ 500	78	4
Total Aluminum – lab (μg/L)	≥ 500	70	3
Sulfate – lab (mg/L)	≥ 50	68	10
Chloride lab (mg/L)	≥ 20	129	50
Total Nitrogen – lab (mg/L)	≥ 2	48	18
Ammonia – lab (mg/L)	≥ 2	151	4
Nitrite – lab (mg/L)	≥ 2	153	44
Total Phosphorus– lab (mg/L)	≥ 0.5	159	14

Generally, initial condition tiers were increased by a quarter- or half-tier if any of the chemistry screening values were violated; although initial condition tiers for some sites were increased by more than a half-tier in cases of chemistry screening value violations of extreme magnitude and/or violations of chemistry screening values for multiple parameters at one site.

Using ESRI ® ArcMap™ 9.2 software and internally available DEP regulated facility GIS data layers, the presence of many other potential sources of anthropogenic disturbance in each sampled watershed was also evaluated and used to adjust initial tier assignments where deemed necessary (Table 3).

Table 3. Screening criteria used to evaluate other potential sources of anthropogenic disturbance to sample sites.

Parameter	Screening value	Disturbance category	Number of samples above/below screening value
% Abandoned mine lands	≥ 5%	Resource extraction	51
% Longwall mine panels	≥ 0%		8
Oil and gas facility density	≥ 5 / mi^2		106
Industrial mineral mining facility density	≥ 0.5 / mi^2		28
Coal mining facility density	≥ 0.5 / mi^2		41
Orphan mine discharge density	≥ 0.01 / mi^2		23
Dam count – large	≥ 1	Hydrologic modification	19
Dam count – medium	≥ 1		119
Dam count – small	≥ 20		22
Dam density – small	≥ 0.5		40
Levee density	≥ 0.01 / mi^2		4
Water allocation density	≥ 0.1 / mi^2		54
Surface water withdrawal density	≥ 0.1 / mi^2		151
Ground water withdrawal density	≥ 2 / mi^2		62
Water pollution control facility discharge density	≥ 1 / mi^2		169
Residual waste facility density	≥ 0.1 / mi^2	Waste facilities	45
Municipal waste facility density	≥ 0.1 / mi^2		155
Captive hazardous waste facitlity density	≥ 0.1 / mi^2		48
Commercial hazardous waste facility density	≥ 0.05 / mi^2		6
Toxic release inventory count	≥ 1	Toxic facilities	236
Land recycling cleanup location count	≥ 1		371
City count	≥ 1	Other	175
Golf course count	≥ 1		79
Erosion and sediment control project density	≥ 1 / mi^2		59
Channel or wetland alteration project density	≥ 1 / mi^2		94
Brownfield count	≥1		49
Fish hatchery count	≥ 1		21

Generally, initial condition tiers were increased by a quarter- or half-tier if any of the anthropogenic disturbance screening values were violated; although initial condition tiers for some sites were increased by more than a half-tier in cases where anthropogenic disturbances were located close to sampling locations and/or if the nature of the facility suggested relatively severe impacts to the sampling location. For example, if a large, constantly discharging wastewater treatment plant or a large dam was located immediately upstream of the sampling location in a small watershed, the initial condition tier was increased more than if a few relatively minor channel alteration projects (e.g., culverts) were located many miles upstream from a sampling location in a larger watershed. Comments recorded in the field by sampling biologists, where available, and site-by-site GIS-based analyses helped to guide this process. In some instances, initial condition tiers were not increased even if some of the anthropogenic

disturbance screening values were violated; this occurred most often when facility sites were located far away from sampling points and/or were considered relatively minor in terms of spatial and/or temporal impact, as discussed above. For example, a site with a golf course located many miles upstream from a sampling site in a large watershed may not have resulted in adjustment to the initial condition tier. Initial condition tiers were also adjusted more than a half-tier in some cases where multiple, relatively severe disturbances were located in the upstream watershed.

It should be emphasized that the anthropogenic disturbance screening values listed in Table 3 were used in a relatively minor role to more accurately and comprehensively assess the conditions at each site, which were driven mainly by land use and physical habitat data, with additional adjustments based on chemistry data. One of the primary reasons this data was even included in the analysis was the spotty nature of associated water chemistry at many sites and the lack of physical habitat data at some sites.

DEP recognizes that the criteria values cited in Table 3 are essentially arbitrary and that if the facilities are (or were) operating within established permit limits, impacts to aquatic life should be minimal or absent. However, the goal of this coarse-level screening process was to identify sampled watersheds with higher levels of human activity – both past and present – that may be (or have been) impacted by sources of disturbance not accounted for in the analysis of land use, physical habitat and water chemistry. All of the facilities listed in Table 3 have the potential to impact stream ecosystems and the screening values were set to identify only those watersheds with the extreme highest values for each parameter in the dataset.

In addition to evaluations of land use, physical habitat, water chemistry and other potential sources of anthropogenic disturbance, condition tier adjustments for each sampled stream segment were made in a few cases based on the attainment or impairment of its ALU. A few sample sites were also adjusted to more disturbed tiers based on the fact that they were sampled as part of effluent dominated stream surveys.

Use of the current ALU attainment status in determining the condition tier of a stream introduces some concern of logical circularity or biased influence from preconceived notions about the reference condition for benthic macroinvertebrate communities (see Stoddard et al. 2006). The ALU attainment status of most streams in Pennsylvania is currently based on the results of DEP's statewide surface water assessment program (SSWAP), which consisted of family-level identification of macroinvertebrate communities and habitat evaluations resulting in a field determination by the investigating biologists as to the attainment or impairment status of the ALU for a stream. Appendix A discusses the SSWAP methods further. The concern of circularity or bias has to do with using the condition of the biotic assemblage to inform classification of sites as reference or stressed. However, as evidenced by the results of the Pennsylvania TALU workshops (see discussion below), numerous experienced biologists thoroughly understand the benthic macroinvertebrate communities in Pennsylvania's wadeable freestone streams and there is a very well-defined idea of what constitutes a "natural" community as opposed to a community under severe

anthropogenic stress. In addition, it is generally agreed that if the ALU of a stream was impaired during SSWAP, it is very likely that the ecosystem was under substantial anthropogenic stress.

Final condition tier assignments placed each sample site into one of four condition tiers: tier A (404 sites); tier B (260 sites); tier C (259 sites); or tier D (352 sites) with tier A representing “reference-quality” sites and tier D representing “stressed” sites (Figure 2). Final condition tiers generally corresponded to the following adjusted initial condition tiers: tier A = 1.00 to 1.50; tier B = 1.75 to 2.50; tier C = 2.75 to 3.50; and tier D = 3.75 to 4.75; although some of the sites lacking physical habitat data did not follow these guidelines exactly, relying more heavily on analyses of water chemistry and other anthropogenic impacts to determine final condition tiers. The set of tier A selected using the steps outlined above include a combination of sites in what Stoddard et al. (2006) describe as the minimally disturbed condition (i.e., free from all but the broadest-scale human disturbance, only minimal pollutant exposure from long-range atmospheric transport), the least disturbed condition (i.e., representing the best existing physical, chemical, and biological habitat given the current state of the landscape), and the best attainable condition (i.e., where the impact on biota of inevitable land use is minimized).

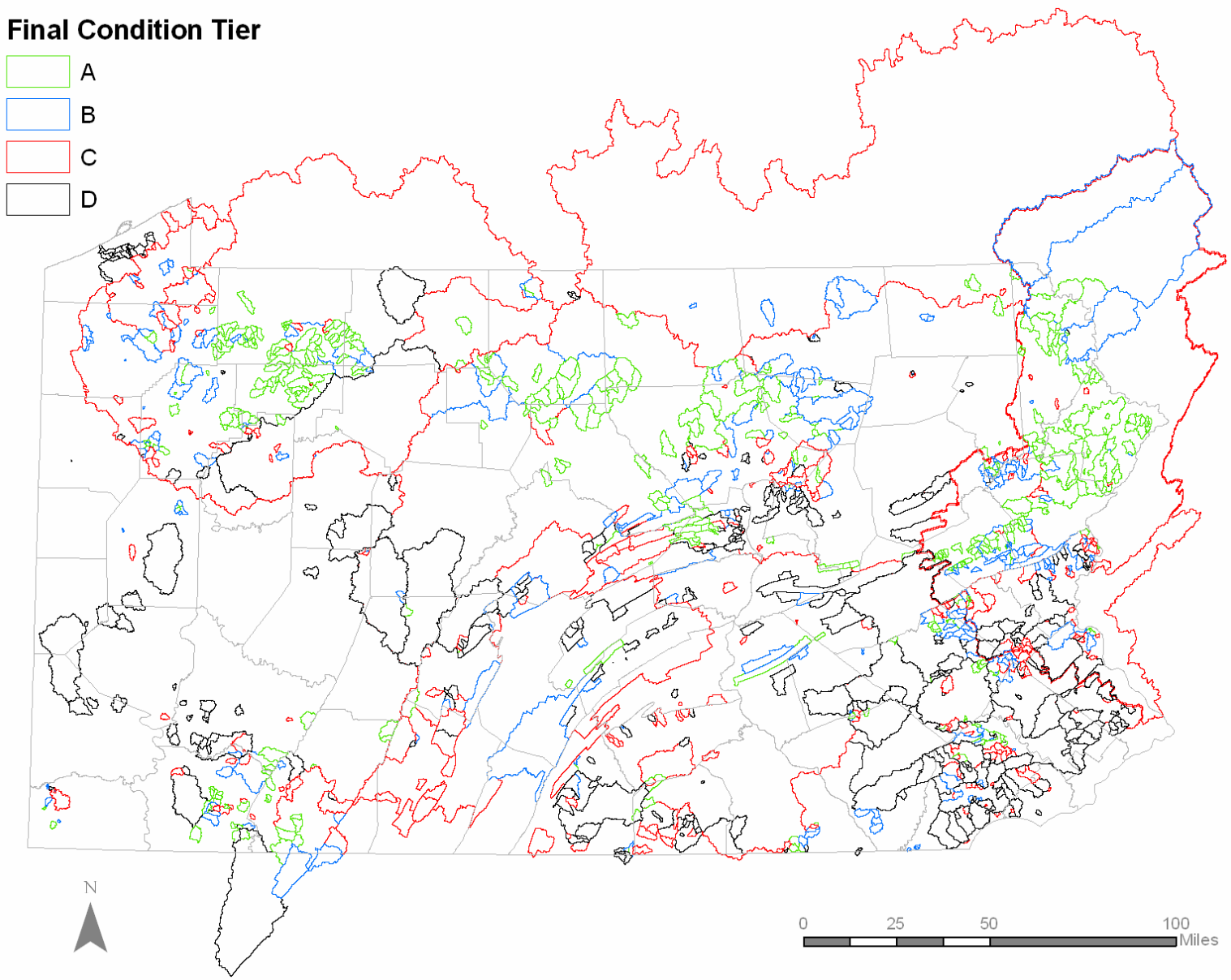

Figure 2. Sample site watersheds (n = 1,275) color coded by final condition tier assignment (with county boundaries).

SITE AND SAMPLE CHARACTERIZATION

The sites sampled were fairly evenly distributed among Pennsylvania's three major drainage basins, with about 37% from the Delaware River basin, 32% from the Susquehanna River basin, 25% from the Ohio River basin and the other 6% of samples distributed about equally between the Great Lake basins and the Potomac River basin.

Most of the 1,275 sites sampled in the dataset were first through third order streams (according to the 1:100,000-scale NHDPlus dataset) draining less than 100 square miles; in fact, 93% of all sites sampled drained less than 100 square miles, and 82% of all sites sampled drained less than 25 square miles (Figure 3). Most tier A sites were situated at higher elevations than tier D sites (Figure 3).

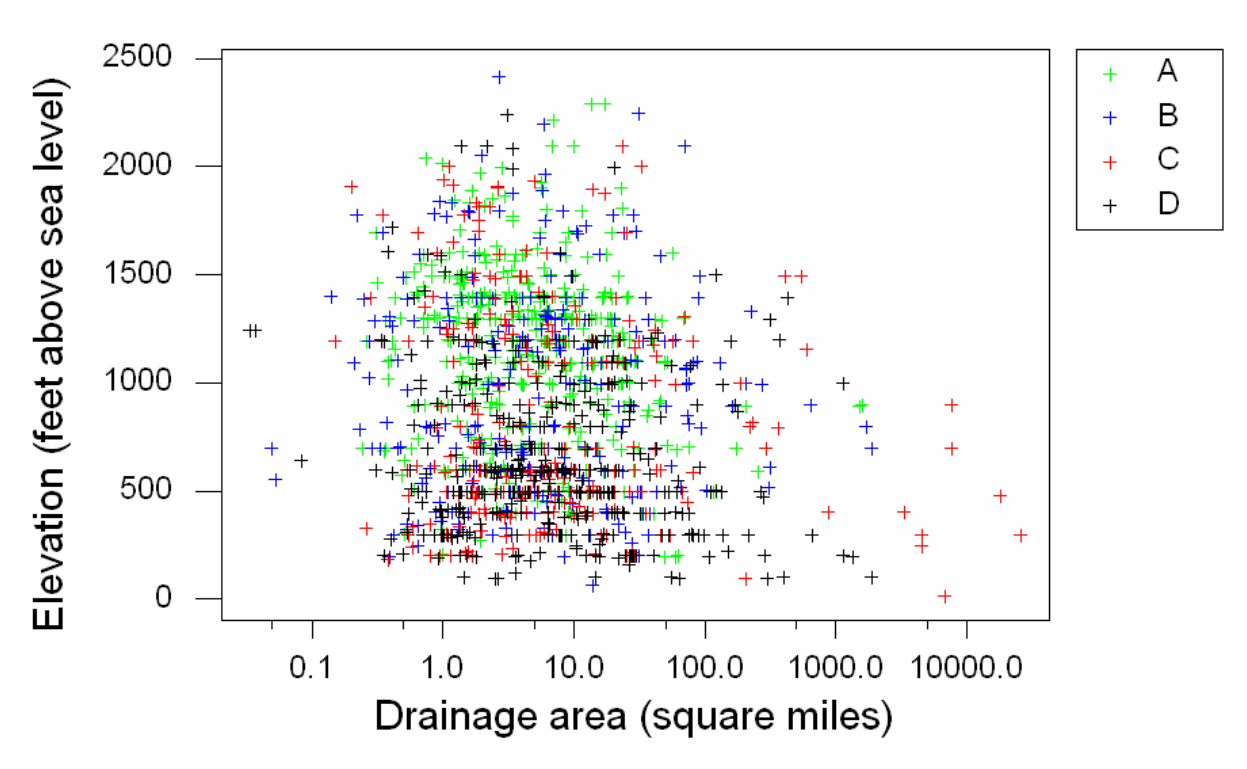

Figure 3. Plot of elevation versus drainage area for the 1,275 sample sites, color coded by final condition tier assignment.

Most samples (58%) were collected during 2006 and 2007, and most samples (58%) were collected during the months of March, April and May, with almost a quarter of the 1,275 samples collected between March and May 2007 (Figure 4).

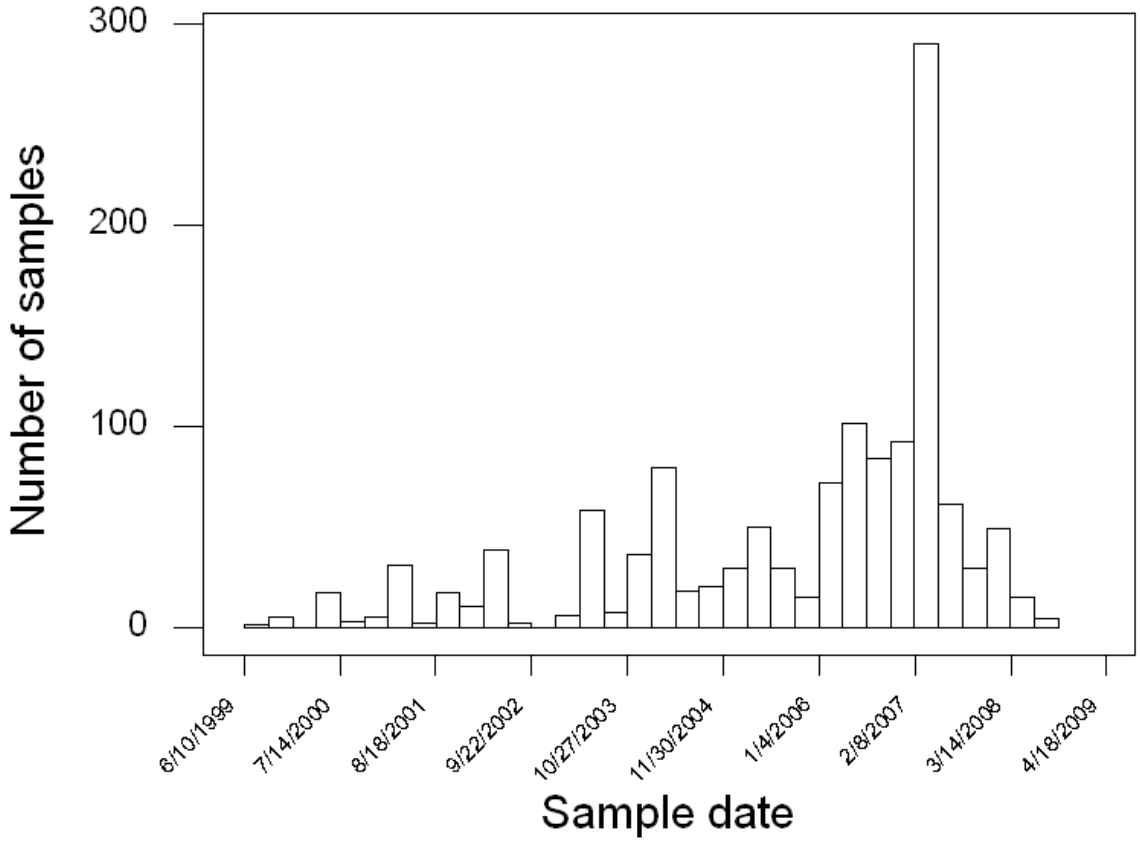

Figure 4. Sampling frequency histogram by date.

SAMPLE CLASSIFICATION

In addition to varying impacts of human activities, natural variation exists among different types of stream ecosystems. In the present context, the goal of a classification scheme is to provide a framework for organizing and interpreting this natural spatial and seasonal variation of complex ecosystems in order to establish the reference condition (Whittaker 1962; Hughes 1995; Barbour et al. 1999). Appropriate ecosystem classification is critical to the reference site concept because it helps determine the spatial and seasonal extent over which a particular biological attribute is applicable (Hughes 1995).

Stream classification identifies relatively homogenous classes of streams among which biological expectations may differ and from which the best and most representative sites should be selected to establish the reference condition (Barbour et al. 1999). Inappropriate classification across heterogeneous classes may result in misrepresentation of the biological condition in certain ecosystem types. For these reasons, the need for some sort of classification scheme that groups streams together that are more similar than others (e.g., true limestone spring streams versus freestone streams) should be carefully evaluated (Hughes 1995). Evaluation of biological attributes that represent structures and functions of the "natural" community represents a critical component of any classificatory analysis of biological data (Hughes 1995). Biological expectations vary in time and space, along with colonizing potential, climate, geology, soils, land use and other factors. An analysis of taxa sampled from various areas and seasons can help identify important classifications for biological expectations (Hughes 1995).

Two multivariate statistical methods were used to evaluate the biological relevance of various potential classification schemes: agglomerative hierarchical cluster analysis (Lance and Williams 1967; Milligan 1989) and non-metric multidimensional scaling, or NMDS (Kruskal and Wish 1978; Ludwig and Reynolds 1988). Both types of analyses, which have been used in similar applications evaluating biological integrity of stream ecosystems (see Barbour et al. 1995; Hawkins and Norris 2000), were performed using SAS ® 9.1 software. The groups defined by the cluster analysis can be thought of as an *a posteriori* classification scheme based solely on characteristics of the biological community, while the other schemes tested were determined *a priori* based on natural variations of physiochemical, biogeographical and/or seasonal characteristics (Barbour et al. 1999).

All sample classification analyses were based on matrices of Bray-Curtis similarity measures (Ludwig and Reynolds 1988) calculated on natural log-transformed proportional abundance of taxa from tier A samples only in order to minimize variation attributable to anthropogenic impacts. Extremely rare taxa (i.e., those found at less than 1.5% of all tier A sites) were not included in the classification analyses. Previous analyses (see Marchant 1999, 2002) suggest that extremely rare taxa are largely unimportant to multivariate analyses, especially when considering only relatively undisturbed sites, because only more commonly encountered taxa can be adequately

characterized in terms of response to environmental variables. In addition, extremely rare taxa are more likely to have been misidentified and could obscure the ability to detect biologically significant differences among sites (Hawkins et al. 2000). One-hundred eleven taxa were found at less than 1.5% percent of all tier A sites; exclusion of these 111 extremely rare taxa left 134 taxa that were included in the classification analyses.

Cluster Analysis

The cluster tree resulting from the SAS ® CLUSTER procedure using the flexible beta method with a beta value of -0.25 (Figure 5) was analyzed at the level of 10 clusters, which explained 25% of the variation in the data. For purposes of the cluster analysis Bray-Curtis similarity measures were converted to distance measures by subtraction from one. The beta value of -0.25 was chosen based on literature (Milligan 1989) and visual inspection of cluster trees constructed using other beta values. A value of -0.25 produced a tree with easily visually distinguishable groupings, as opposed to other values that tended to produce overly detailed groups (more positive beta values) or overly simplified groups (more negative beta values).

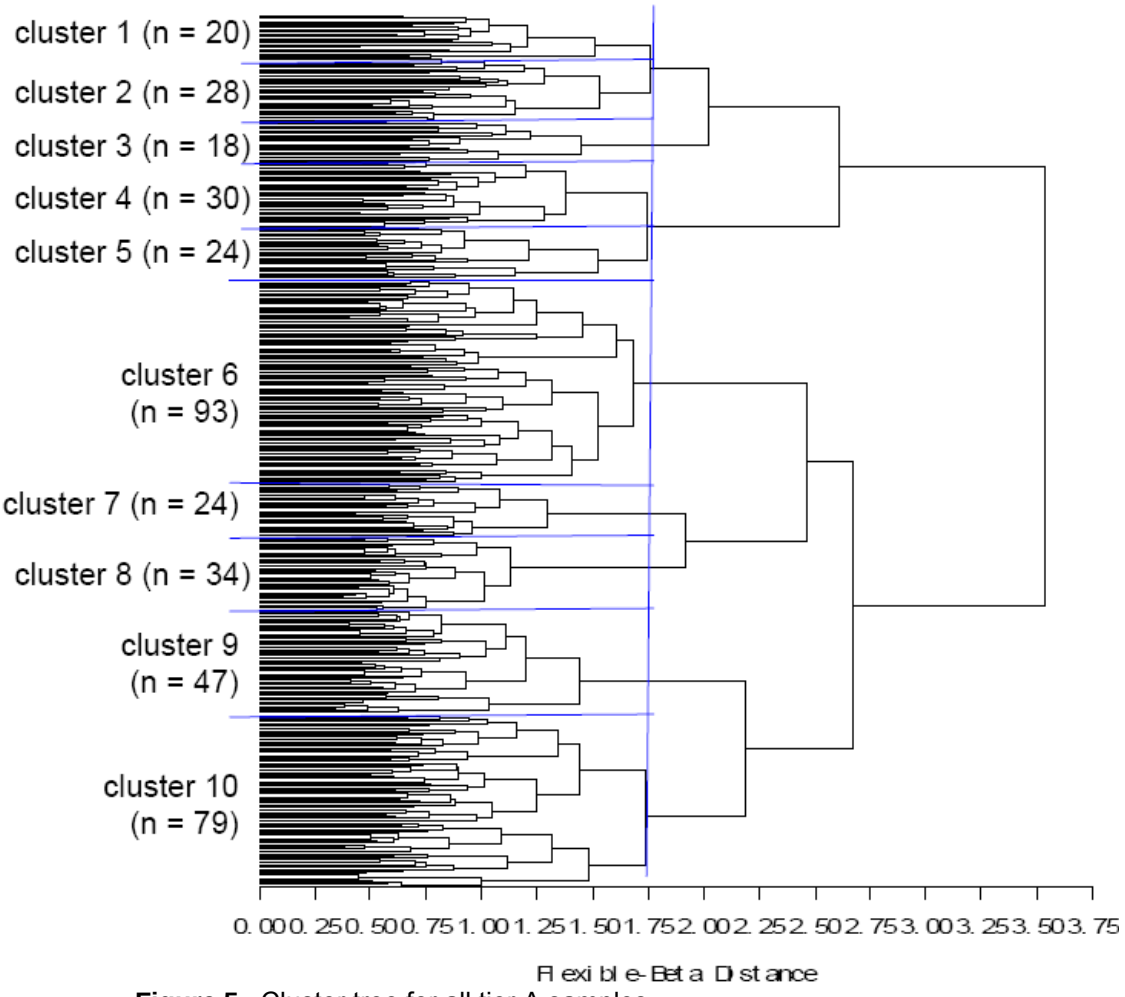

Figure 5. Cluster tree for all tier A samples.

Graphical analyses of the clustered samples by assorted variables (i.e., drainage area, elevation, geographic location, sampling date, drainage basin) are presented in Appendix B. The major noticeable patterns in the clustered samples were seen for drainage area and sampling date, as well as geographic location (see Appendix B). Clusters 4 and 5 drained noticeably larger sites and clusters 7 and 8 drained noticeably smaller sites (Figure 6, Appendix B). Clusters 1, 2 and 3 contained mostly samples taken between June and December, while clusters 7 – 10 contained mostly samples from January to April; clusters 4, 5 and 6 contained a mix of sampling dates, with clusters 4 and 5 containing samples mostly from May to December and cluster 6 containing mostly May samples (see Appendix B).

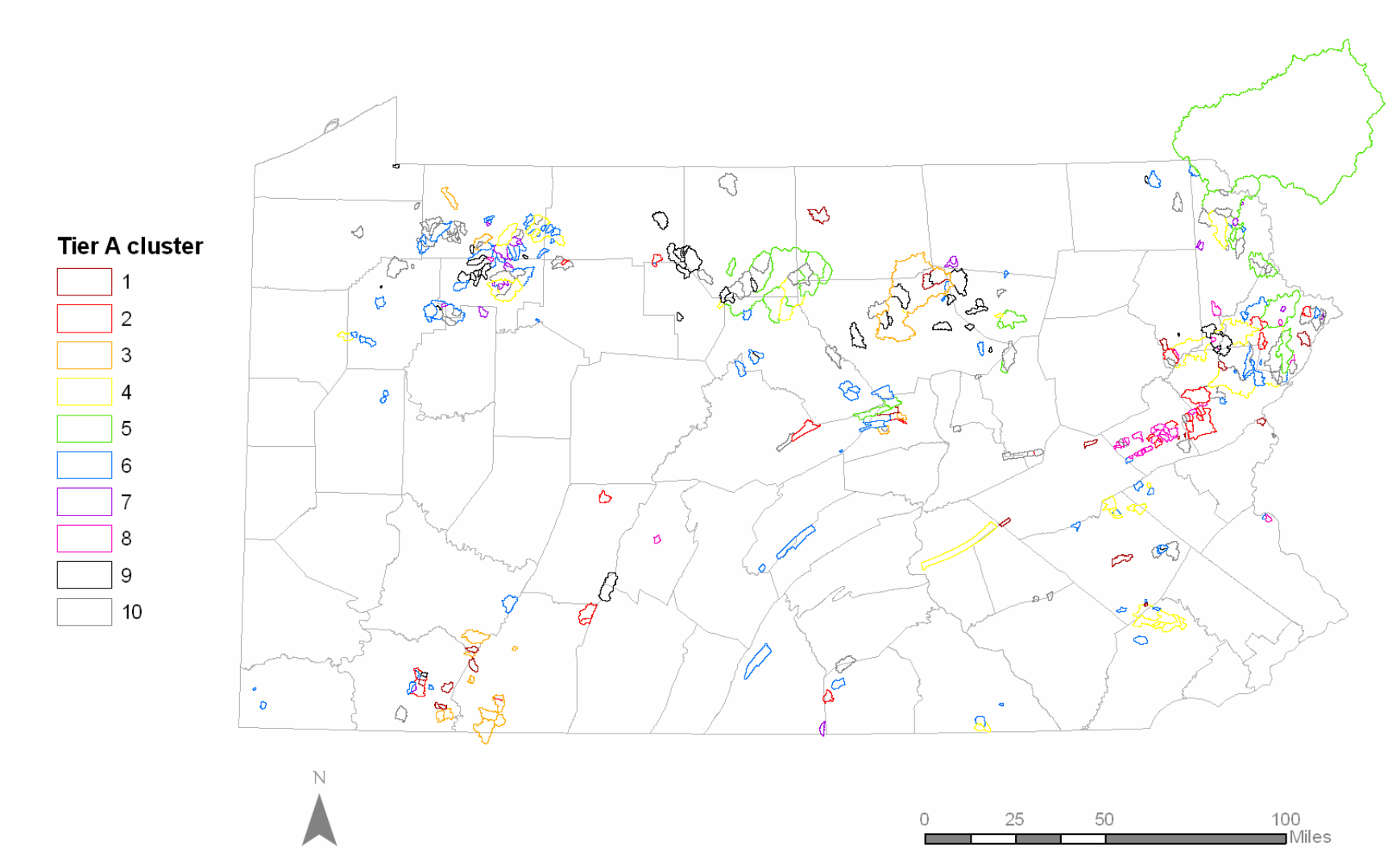

Figure 6. Color coded map of tier A samples by cluster number (with county boundaries).

Discriminant function analysis (Fisher 1936; Hand 1981), another multivariate statistical technique, was used to further explore the results of the cluster analysis. Basically, this technique can be used to determine how much various parameters contribute to the classifications resulting from the cluster analysis. Nonparametric linear discriminant functions based on the four nearest-neighbors method using six variables (drainage area, Julian day, latitude, longitude, elevation and gradient class) resulted in decent concordance with the cluster groups (55% of reference sites classified into their original cluster group). This analysis was performed with five cluster groups instead of ten to limit the number of groups and for purposes of comparison with an earlier iteration of this project; general patterns in the tier A samples were still apparent at the level of five clusters (Figure 5, Appendix B).

Julian day had the strongest coefficient value for the first canonical function by far, followed by latitude, drainage area and gradient class (Table 4). Similarly, drainage area had the strongest coefficient value for the second canonical function by far, followed by gradient class, latitude and elevation (Table 4). These results suggest primary importance of sampling date and drainage area in discriminating among the cluster groups, with secondary influences of latitude, gradient class and elevation. Similar patterns can be observed in the more detailed graphical analysis of cluster groups in Appendix B.

Table 4. Standardized coefficients for selected variables resulting from a canonical discriminant function analysis.

Parameter	Canonical function 1	Canonical function 2	Canonical function 3	Canonical function 4
Drainage area (square miles)	0.43	0.72	0.31	0.35
Julian day	0.89	-0.07	-0.33	-0.09
Latitude	-0.45	0.46	-0.18	0.20
Longitude	0.05	0.03	0.60	-0.70
Elevation	-0.07	-0.38	0.09	0.71
Gradient class	-0.32	-0.51	-0.38	0.09
Eigenvalue	0.64	0.44	0.11	0.04
Cumulative proportion	0.53	0.88	0.97	1.00

NMDS

Appendix C presents NMDS ordination plots classified according to a variety of classification schemes (e.g., sampling season, drainage area, ecoregions, physiographic provinces). The NMDS analysis generally confirmed the patterns observed in the cluster analysis (Figure 7), showing the strong influence sampling season and drainage area exert on the tier A benthic macroinvertebrate community distributions (see Appendix C). The "badness-of-fit," or "final stress," criterion for the two-dimensional NMDS was 0.22.

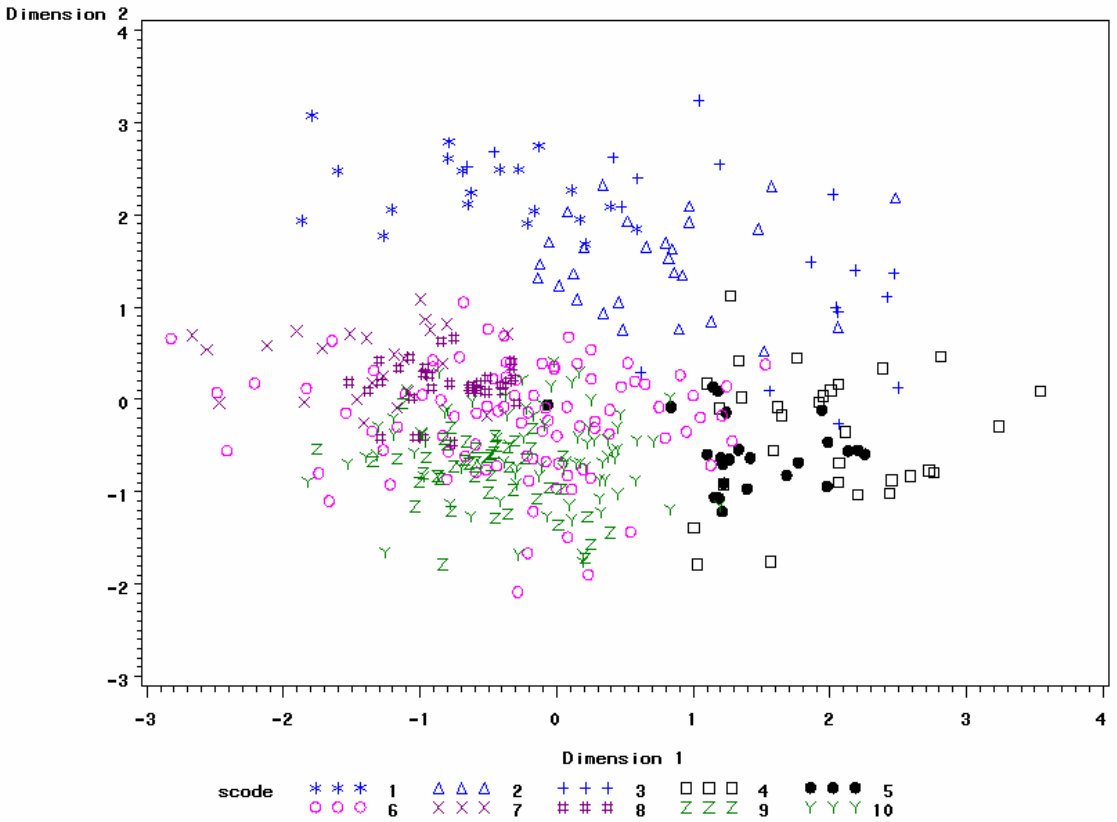

Figure 7. Plot of first two NMDS dimensions for tier A samples, classified by the 10 cluster groups from the cluster analysis.

Methods described in Van Sickle and Hughes (2000) – aided by MEANSIM, Version 6.0 software (Van Sickle 1998) – were used to quantify classification strengths of the various classification schemes (see Hawkins and Norris 2000). The classification strength of each scheme was quantified through two primary parameters: $\mathbf{W_{bar}}$, measuring the *within class similarity* and $\mathbf{B_{bar}}$, measuring the *between class similarity*. Strong classification schemes minimize similarity between classes (i.e., maximize between class variation) and maximize similarity within classes (i.e., minimize within class variation), resulting in lower B_{bar}/W_{bar} ratios and higher $W_{bar} - B_{bar}$ values than weaker classification schemes.

Table 5. Classification strengths of various classification schemes tier A reference samples. After Table 1 in Van Sickle and Hughes (2000).

Classification Scheme	Number of Classes	Natural Log Transformed Taxa Abundance (rare taxa not included)		
		Wbar - Bbar (%)	Bbar / Wbar	W bar (%)
ANNUAL SEASONS				
Jan - May, Jun - Sep, Oct - Dec	3	**6.90**	**0.856**	48.0
Months	12	**6.93**	**0.861**	49.9
Jan - Apr, May - Jul, Aug - Sep, Oct - Dec	4	**6.51**	**0.869**	49.5
01 Jan - 15 May, 16 May - 30 Jun, 01 Aug - 15 Oct, 16 Oct - 31 Dec	4	**5.84**	**0.869**	44.6
DRAINAGE AREA				
<50 or >50	2	**7.86**	**0.815**	42.6
0-1, 1-5, 5-10, 10-25, 25-50, 50-100, 100-300, >1,000	8	**4.17**	**0.909**	46.0
Stream order	5	**3.85**	**0.914**	44.6
ANNUAL SEASONS + DRAINAGE AREA				
Jan - May, Jun - Sep, Oct – Dec and < 50 or > 50	5	**6.00**	**0.859**	42.6
ECOREGIONS				
Bioregions from summer 2-kick protocol (Plateau, Mountains, Piedmont)	7	**2.29**	**0.944**	41.2
Bailey - Provinces (Eastern Broadleaf Forest, Laurentian Mixed Forest, Central Appalachian Broadleaf-Coniferous Forest-Meadow, Adirondack-New England Mixed-Coniferous Forest-Apline Meadow)	8	**1.76**	**0.958**	41.8
TNC (Western Allegheny Plateau, High Allegheny Plateau, Central Appalachian Forest, Lower New England / Northern Piedmont)	8	**1.74**	**0.958**	41.5
Omernik - Level 2 (Atlantic Highlands, Mixed Wood Plains, Southeastern USA Plains, Appalachian Forests)	8	**1.52**	**0.963**	41.5
BASINS				
River Basin (Allegheny, Monongahela, Ohio, Upper Susquehanna, West Branch Susquehanna, Lower Susquehanna, Potomac, Upper Delaware, Lower Delaware)	9	**3.85**	**0.912**	43.6
Drainage (Mid-Atlantic, Ohio River)	2	**2.13**	**0.949**	41.5
PHYSIOGRAPHY and GEOLOGY				
Rock type	8	**1.96**	**0.953**	41.5
Physiographic provinces	6	**0.78**	**0.981**	41.4
OTHER				
Clusters from cluster analysis (10)	10	**10.90**	**0.801**	54.7
Clusters from cluster analysis (5)	5	**8.40**	**0.830**	49.4
Slope class	3	**3.74**	**0.916**	44.7

Compared to other classification strength analyses (Van Sickle and Hughes 2000), all of the classification schemes tested resulted in fairly low classification strengths, with the groups from the cluster analysis having the highest measures of classification strengths. However, sampling season and drainage area classifications produced strong classification schemes compared to the other schemes tested here (Table 5, Appendix C).

METRIC ANALYSIS AND INDEX DEVELOPMENT

A biological metric quantifies some measurable characteristic of the biota that changes in some predictable way with increased anthropogenic stress (Barbour et al. 1995). Metrics allow us to measure meaningful indicator attributes in assessing the biological condition of sample sites (Barbour et al. 1999). Vast arrays of metrics have been tested in various applications developing indices of biotic integrity for a variety of aquatic assemblages, including benthic macroinvertebrates (Barbour et al. 1995). The utility of each metric is based on a hypothesis about the predictable relationship between the biological response measured by that metric and ecosystem stress caused by human impacts (Barbour et al. 1995; Yoder and Rankin 1995).

The Multimetric Index

Most water resource agencies in the United States use a multimetric approach to developing indices of biological integrity (Barbour et al. 1999). This approach utilizes a suite of metrics that individually measure diverse biological attributes and exhibit various responses to different stressors. A major benefit of the multimetric approach is the ability to incorporate information from a number of metrics that, when integrated into a single measure, or index, can provide a meaningful indicator of overall biological condition (Barbour et al. 1995). Such an index helps to increase sensitivity to a broad range of ecosystem stressors and to minimize any weaknesses or limitations that each underlying metric may have if used individually. For example, some metrics are sensitive across a broad range of biological conditions and other metrics are only sensitive in part of the range. Metrics that exhibit detectable responses to changing disturbance conditions are important for indicating comparability to – or departure from – the established reference biological condition. Overlap in the ranges of sensitivity of individual metrics helps strengthen conclusions regarding biological condition reached using an integrative, multimetric index approach (Barbour et al. 1995).

Candidate Metrics

Ideally, evaluation of candidate metrics should result in selection of metrics that: (1) are based in well-understood ecological principles relevant to the biological community in the type of water body being studied as well as to sampling methods and assessment objectives; (2) respond to anthropogenic stress in a predictable manner; (3) have responses to stressors that can be distinguished from natural variation and that can discriminate along a gradient of anthropogenic stress; (4) are environmentally benign to measure; and (5) are cost-effective to sample (Barbour et al. 1995). The most useful indices of biological integrity incorporate metrics based on sound ecological principles and representing diverse aspects of structure, composition, individual health, and/or processes of the biological community. Such metrics quantify expectations defined by the reference condition and can serve as the foundation of a sound, integrated assessment of biological condition (Barbour et al. 1995).

A number of major classes of attributes have been generally defined for metrics applied to benthic macroinvertebrate communities: taxonomic richness; community composition; pollution tolerance; trophic guild; behavior or motility habit; and life cycle (Barbour et al. 1999). Candidate metrics considered in this analysis generally fit into one of these major categories, although some metrics measure aspects of two or more of these major classes (Table 6). No measures of individual condition were considered because DEP does not routinely assess individual condition of benthic macroinvertebrates due to the difficultly and considerable time involved.

Table 6. Candidate metrics considered during this project. Proportional taxa richness metrics measure the taxa richness of a particular type of taxa (e.g. Ephemeroptera taxa) divided by the total taxa richness.

Taxonomic Group	Taxa Richness	Proportional Taxa Richness	% Individuals	Other	Notes	Expected Response to Increasing Anthropogenic Stress
Total Taxa	X					Decrease
Special Protection Indicator Taxa	X					Decrease
Ephemeroptera (Mayfly) Taxa ***	X	X	X			Decrease
Plecoptera (Stonefly) Taxa	X	X	X			Decrease
Trichoptera (Caddisfly) Taxa ***	X	X	X			Decrease
Ephemeroptera + Plecoptera + Trichoptera Taxa***	X	X	X			Decrease
BCG Attribute I Taxa	X	X	X			Decrease
BCG Attribute II Taxa	X	X	X			Decrease
BCG Attribute III Taxa	X	X	X			Decrease
BCG Attribute I + II + III Taxa	X	X	X			Decrease
BCG Attribute IV Taxa	X	X	X			Increase
BCG Attribute V Taxa	X	X	X			Increase
BCG Attribute IV + V + VI Taxa	X	X	X			Increase
(BCG Attribute I + II + III Taxa) / (BCG Attribute IV + VI + VI Taxa)	X	X	X			Decrease
PTV 0 – 5 Taxa	X	X	X			Decrease
PTV 0 – 4 Taxa	X	X	X			Decrease
PTV 0 – 3 Taxa	X	X	X			Decrease
PTV 0 – 2 Taxa	X	X	X			Decrease
PTV 5 – 10 Taxa	X	X	X			Increase
PTV 6 – 10 Taxa	X	X	X			Increase
PTV 7 – 10 Taxa	X	X	X			Increase
PTV 8 – 10 Taxa	X	X	X			Increase
Hilsenhoff Biotic Index				X	Number of individuals weighted by PTV score	Increase
BCG Index				X	Number of individuals weighted by BCG Attribute	Increase
Beck's Index				X	Taxa richness weighted by PTV score or BCG Attribute – Multiple versions tested	Decrease
Predator Taxa	X	X	X			Decrease
Shredder Taxa	X	X	X			Decrease
Filter-Collector Taxa	X	X	X			Increase
Collector-Gatherer Taxa	X	X	X			Increase

Taxonomic Group	Taxa Richness	Proportional Taxa Richness	% Individuals	Other	Notes	Expected Response to Increasing Anthropogenic Stress
Scraper Taxa	X	X	X			Increase
Dominant Taxa			X			Increase
Functional Feeding Group Similarity to Reference Community				X	Reference community defined based on samples from EV stream sites	Decrease
Shannon Diversity				X	Distribution of individuals among taxa	Decrease
Functional Feeding Group Shannon Diversity				X	Distribution of individuals among functional feeding groups	Decrease
Non-Insecta Taxa			X			Increase
Hirudinea Taxa			X			Increase
Oligochaeta Taxa			X			Increase
Polychaeta Taxa			X			Increase
Diptera Taxa ***	X		X			Increase/ Decrease***
Chironomidae Taxa			X			Increase (?)
Simuliidae Taxa			X			More a seasonal metric than a stress metric
Capniiade Taxa			X		Winter Plecoptera	Decrease
Taeniopterygidae Taxa			X		Winter Plecoptera	Decrease
Elmidae Taxa ***	X		X			Increase
Corbiculidae Taxa			X			Increase
Hydropsychidae Taxa ***	X		X			Increase
Hydropsyche + Cheumatopsyche			X			Increase
Ratio of Tolerant Hydropsychidae to Total Trichoptera Taxa			X			Increase
Ratio of Hydropsyche + Cheumatopsyche to Total Trichoptera Taxa			X			Increase
Isopoda + Gammaridae + Ephemerella			X		Indicator of limestone influence	More an indicator of limestone influence than a stress metric

*** these metrics were computed using all taxa and using only certain sensitive and/or tolerant taxa

Discrimination Efficiency

The ability of each candidate metric to discriminate between reference and stressed samples was quantified as discrimination efficiency. For metrics expected to decrease in value with increasing anthropogenic stress, or negative-response metrics, the following equation was used to calculate the discrimination efficiency:

$$D.E.\ (\%) = n_{tierD<\%tierA} / n_{tierDtotal} * 100$$

where D.E. = the discrimination efficiency, $n_{tierD<\%tierA}$ = the number of tier D samples with metric values less than some percentile value of all tier A samples, and $n_{tierDtotal}$ = the total number of tier D samples. Discrimination efficiencies for negative-response metrics were evaluated at $n_{tierD<\%tierA}$ values corresponding to the 25^{th}, 10^{th}, 5^{th} and 1^{st} percentiles as well as the minimum of tier A samples.

For metrics expected to increase in value with increasing stress, or positive-response metrics, the following equation was used to calculate the discrimination efficiency:

$$D.E.\ (\%) = n_{tierD>\%tierA} / n_{tierDtotal} * 100$$

where D.E. = the discrimination efficiency, $n_{tierD>\%tierA}$ = the number of tier D samples with metric values greater than some percentile value of all tier A samples, and $n_{tierDtotal}$ = the total number of tier D samples. Discrimination efficiencies for positive-response metrics were evaluated at $n_{tierD>\%tierA}$ values corresponding to the 75^{th}, 90^{th}, 95^{th} and 99^{th} percentiles as well as the maximum of tier A samples.

Metrics with minimal or no overlap between the tier A (i.e., reference) and tier D (i.e., stressed) site distributions (i.e., high discrimination efficiencies) can be considered strong, predictable discriminators between reference and stressed conditions and provide the most confidence for assessing the biological condition of unknown sites (Barbour et al. 1999). Metrics with high discrimination efficiencies (Figure 8) were selected for further evaluation; due to the large number of metrics evaluated, discrimination efficiencies are presented here for only the six metrics selected for inclusion in the final IBI (Table 7) – discrimination efficiencies for other candidate metrics are available upon request.

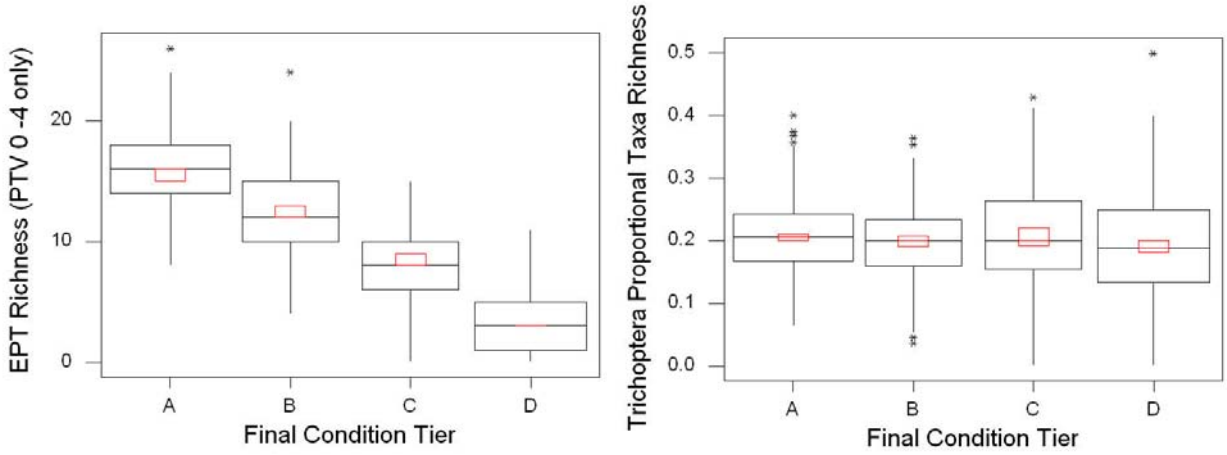

Figure 8. Example boxplots of metric values against the four condition tiers for two candidate metrics. Sensitive EPT Richness, counting only EPT taxa with pollution tolerance values (PTVs) of 0 to 4 (left) shows a very high discrimination efficiency among condition tiers while Trichoptera Proportional Taxa Richness (right) shows a very low discrimination efficiency among condition tiers.

Table 7. Discrimination efficiency for selected core metrics. (PTV = pollution tolerance value)

Candidate Metric	Expected Response to Increasing Anthropogenic Stress	Discrimination Efficiency @ __ percentile of tier A samples			
		10th	25th	90th	75th
Total Taxa Richness	Decrease	91.2%	97.7%	---	---
Ephemeroptera + Plecoptera + Trichoptera Taxa Richness (PTV 0 – 4 only)	Decrease	100.0%	100.0%	---	---
Beck's Index – version 3	Decrease	100.0%	100.0%	---	---
Shannon Diversity	Decrease	84.0%	97.7%	---	---
Hilsenhoff Biotic Index	Increase	---	---	92.6%	95.7%
% Sensitive Individuals (PTV 0 – 3 only)	Decrease	92.9%	95.7%		

Metric Correlations

In order to help select strongly discriminating metrics while reducing the number of metrics relating redundant information, metric correlations were analyzed for all metrics with high discrimination efficiencies. Due to the large number of metrics analyzed, correlations are presented here only for the six metrics selected for inclusion in the final IBI (Table 8) – correlations for other candidate metrics are available upon request.

Table 8. Pearson correlation (r) values for the six selected core metrics. (PTV = pollution tolerance value)

Metric	Total Taxa Richness	EPT Taxa Richness (PTV 0 – 4 only)	% Sensitive Individuals (PTV 0 – 3 only)	Hilsenhoff Biotic Index	Shannon Diversity	Beck's Index, version 3
Total Taxa Richness	1.000					
EPT Taxa Richness (PTV 0 – 4 only)	0.865	1.000				
% Sensitive Individuals (PTV 0 – 3 only)	**0.484**	**0.717**	1.000			
Hilsenhoff Biotic Index	-0.520	**-0.733**	**-0.934**	1.000		
Shannon Diversity	0.857	**0.756**	0.438	**-0.508**	1.000	
Beck's Index, version 3	0.765	0.926	0.729	-0.751	0.674	1.000

The correlation between the EPT Taxa Richness (counting only taxa with pollution tolerance values, or PTVs, 0 – 4) metric and the Beck's Index, version 3 metric was fairly high (r = 0.926), as was the correlation between the Hilsenhoff Biotic Index metric and the % Sensitive Individuals (PTV 0 – 3) metric (r = -0.934). However, scatterplots of the relationship between these pairs of metrics (Figure 10) revealed enough variation that all were retained for inclusion in the final IBI.

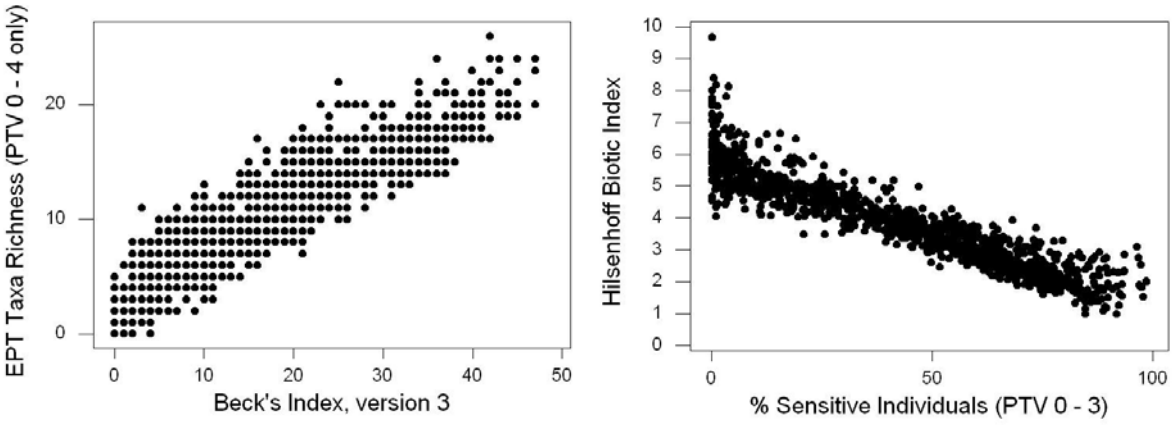

Figure 10. Scatterplots of two pairs of metrics with high Person correlation coefficients. All four of the metrics were retained for inclusion in the final IBI because, despite the high correlations, there is still substantial variation in the metric values to provide useful information. In the Hilsenhoff Biotic Index vs. % Sensitive Individuals plot (right) note especially the range of the Hilsenhoff Biotic Index metric at the low end of the % Sensitive Individuals metric.

A number of different metric combinations were evaluated during index development. Based on discrimination efficiencies, correlation matrix analyses and other index performance characteristics discussed below, the following six metrics were selected for inclusion as core metrics in the multimetric index (Appendix D shows examples of the six core metric and index calculations for a sample and Appendix E contains the pollution tolerance values for all taxa in this dataset).

Total Taxa Richness

This taxonomic richness metric is a count of the total number of taxa in a sub-sample. Generally, this metric is expected to decrease with increasing anthropogenic stress to a stream ecosystem, reflecting loss of taxa and increasing dominance of a few pollution-tolerant taxa. Other benefits of including this metric include its common use in many biological monitoring and assessment programs in other parts of the world as well as its ease of explanation and calculation.

Ephemeroptera + Plecoptera + Trichoptera Taxa Richness (PTV 0 – 4 only)

This taxonomic richness metric is a count of the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) in a sub-sample – common names for these orders are mayflies, stoneflies, and caddisflies, respectively. The aquatic life stages of these three insect orders are generally considered sensitive to, or intolerant of, pollution (Lenat and Penrose 1996); in fact, this metric only counts EPT taxa with pollution tolerance values (PTVs) of 0 to 4, excluding a few of the most tolerant mayfly and caddisfly taxa. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of taxa from these largely pollution-sensitive orders. This metric has a history of use across the world and is relatively easy to use, explain and calculate (Lenat and Penrose 1996).

Beck's Index, version 3

This taxonomic richness and tolerance metric is a weighted count of taxa with PTVs of 0, 1, or 2. The name and conceptual basis of this metric are derived from the water quality work of William H. Beck in Florida (Beck 1955). This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of pollution-sensitive taxa. It should be noted that the version of the Beck's Index metric used for this project, although similar in name and concept, differs slightly in its calculation from the Beck's Index used in DEP's multihabitat protocol for assessing biological condition of low gradient pool-glide type streams (see Appendix D for calculation details).

Shannon Diversity

This community composition metric measures taxonomic richness and evenness of individuals across taxa of a sub-sample. This metric is expected to decrease in values with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive taxa and increasing dominance of a few pollution-tolerant taxa. The name and conceptual basis for this metric are derived from the information theory work of Claude Elwood Shannon (Shannon 1968).

Hilsenhoff Biotic Index

This community composition and tolerance metric is calculated as an average of the number of individuals in a sub-sample, weighted by PTVs. Developed by William Hilsenhoff, the Hilsenhoff Biotic Index (Hilsenhoff 1977, 1987, 1988; Klemm et al. 1990) generally increases with increasing ecosystem stress, reflecting increasing dominance of pollution-tolerant organisms.

Percent Sensitive Individuals (PTV 0 – 3)

This community composition and tolerance metric is the percentage of individuals with pollution tolerance values of 0 to 3 in a sub-sample and is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive organisms.

These six metrics all exhibited a strong ability to distinguish between reference and stressed conditions. In addition, these six metrics measure different aspects of the biological communities represented by the sub-samples, and when used together in a multimetric index, they provide a solid foundation for assessing the biological condition of benthic macroinvertebrate assemblages in Pennsylvania's wadeable freestone stream ecosystems. It should be re-emphasized that a number of different metric combinations were evaluated during index development and that this combination of metrics provided the best performance characteristics, which are further discussed below.

The selected six metrics do not include a metric that directly utilizes the functional feeding group assignment of each taxon. A functional feeding metric was not included in the multimetric index for a number of reasons, primarily because of the difficulty predicting how function feeding metrics respond to different anthropogenic stressors and because natural changes are expected in the distribution of organisms among functional feeding groups with increasing drainage area and associated changes in a stream's trophic dynamics (Vannote et al. 1980); these factors limit the range of applicability of functional feeding metrics to certain stream sizes; further, difficulties with proper assignment of taxa to functional feeding groups contribute to the unreliability of these metrics.

Index Development

An index is simply a means to integrate information from various measures of biological integrity, or various metrics (Barbour et al. 1999). In order to compare and combine sundry measures (e.g., percentage of individuals, counts of taxa, unitless numbers) of biological condition in a meaningful manner, it is necessary to standardize metrics with some mathematical transformation that results in a logical progression of values (Barbour et al. 1995).

Barbour et al. (1999) recommend using a composite of sites representing a gradient of biological conditions (e.g., natural to severely degraded) in the metric standardization and index development process to calibrate the index to a range of biological conditions. As detailed in Appendix D, the one selected core metric that increases in value with increasing anthropogenic stress (i.e., the Hilsenhoff Biotic Index) was standardized to the 5th percentile of metric scores for all samples (i.e., tier A, tier B, tier C and tier D). Core metrics that decrease in value with increasing stress (i.e., total taxa richness, EPT taxa richness, % sensitive individuals, Shannon diversity, Beck's Index) were standardized to the 95th percentile of metrics scores for all samples. The resulting values for any standardized core metric value were set to a maximum value of 1.00 (see Appendix D), with values closer to zero corresponding to increasing deviation from the expected reference condition and progressively higher values corresponding more closely to the biological reference condition (Barbour et al. 1995). This approach establishes upper bounds on the expected condition and moderates effects of metrics that may respond in some manner other than a monotonic response to stress. The adjusted standardized metric values for the six core metrics were averaged and multiplied by 100 to produce an index score ranging from 0 to 100. This number represents the multimetric index of biological integrity (IBI) score for a sample.

As noted in the sample classification section above, benthic macroinvertebrate communities in reference-quality wadeable freestone streams in Pennsylvania showed noticeable variation with annual seasons and drainage area. For this reason, the response of each of the selected six core metrics was evaluated in terms of annual seasons and drainage area. Plots of each of the six core metrics against drainage area and Julian sampling day are presented in Appendix E.

For all six core metrics, a noticeable decline (or increase, in the case of the positive-response Hilsenhoff Biotic Index) in metric scores is apparent in samples from larger systems – even within condition tier types, especially within condition tiers A and B. The effect of drainage area is more pronounced for some metrics (e.g., Hilsenhoff Biotic Index, % Sensitive Individuals) than for others (e.g., Shannon diversity).

Similarly, all six core metrics show some decline during late spring, summer and early autumn months (i.e., late May – early October). Again, this effect is more pronounced for some metrics (e.g., EPT Taxa Richness, % Sensitive Individuals) than for others (e.g., Shannon diversity) and is most noticeable in samples from condition tier A and B sites.

INDEX PERFORMANCE EVALUATION

Biological Condition Discrimination

The range of IBI scores for the tier A, B, C and D sample types exhibited distinguishable separation among the four sample types (Figure 11).

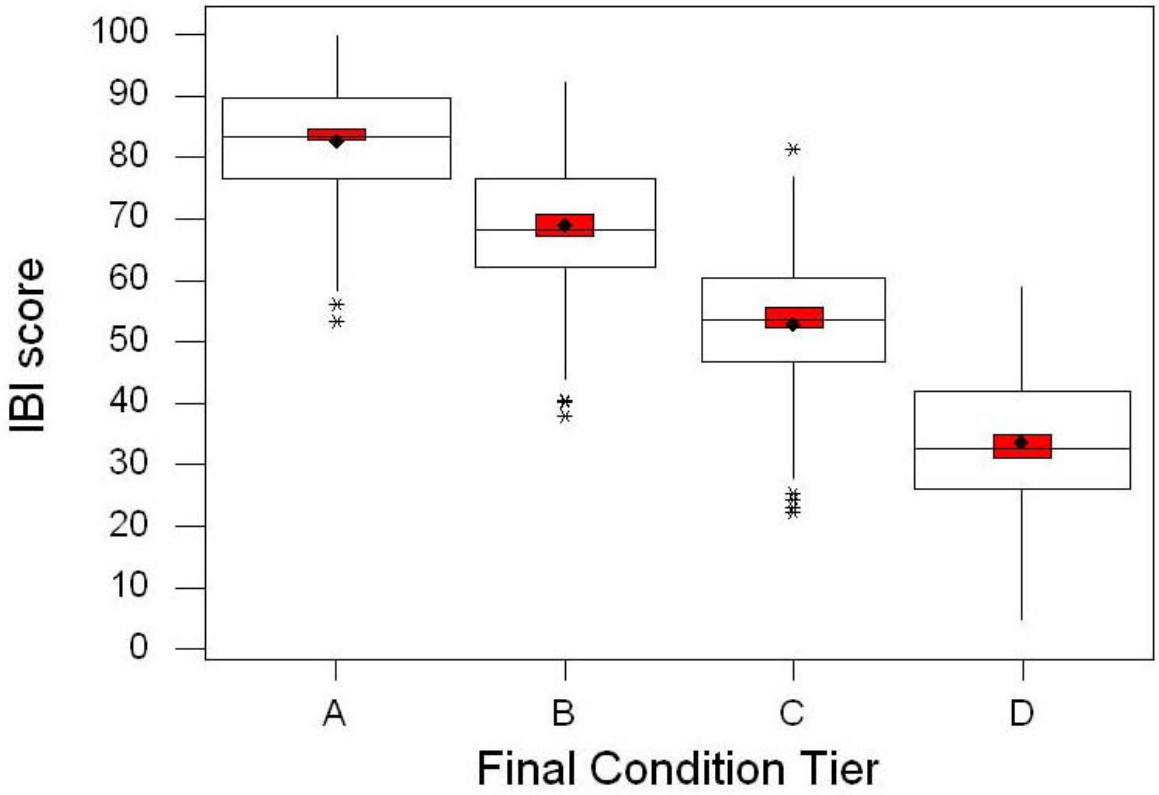

Figure 11. Distribution of IBI scores for each condition tier. Unfilled boxes represent interquartile ranges and are directly proportional to the number of samples. Filled red boxes represent 95% confidence intervals about the median. Diamond markers represent mean values and * symbols represent statistical outliers.

The IBI exhibited excellent discrimination efficiency among tier types. In fact, only five out of 404 total tier A samples (1.2%) scored lower than the highest scoring tier D sample. The ability of the IBI to differentiate among the four pre-established abiotic condition tiers strongly supports its utility in measuring the biological condition of benthic macroinvertebrate communities in Pennsylvania's wadeable freestone streams

Appendix G includes plots of IBI score versus a number of physiochemical parameters, some of which can be used as individual surrogate measures of ecosystem stress (land use, physical habitat, water chemistry). Results from a number of studies (see Novotny 2004) note that relationships between biotic indices and individual surrogate measures of ecosystem stress can be of some value in understanding effects of diffuse pollution

and other stresses on biological integrity, but that these relationships should be interpreted with care.

Intrasite Spatial Variability

Duplicate biological samples were taken at 40 sites and triplicate samples were taken at one site on the same day to provide insight into methodological intrasite spatial variability. Only one sample from each of these sites was used in the IBI development process, but analysis of all the replicate samples can provide an estimate of IBI intrasite spatial precision.

Results of an analysis of variance (ANOVA) on the intrasite, same-day replicated sample data with site as a factor provides an estimate of variation for each set of replicated samples (Table 9). Individual metric values used in the ANOVA procedures were normalized and adjusted as described above and in Appendix D. The ANOVA mean square error (MSE) provides an estimate of within site standard deviation and can be used to calculate confidence intervals around a score. The one-tailed 90% confidence intervals in Tables 9, 10, and 12 were calculated according to the following equation:

$$\text{One-tailed 90\% Confidence Interval} = 1.282 \times [(\text{ANOVA MSE})^{0.5} / (\text{number of samples})^{0.5}]$$

Table 9. Results from a one-way ANOVA with site as a factor performed on the intrasite, same-day replicated sample data.

Metric	ANOVA MSE	Square Root of ANOVA MSE	One-tailed 90% Confidence Interval		
	estimates of within group standard deviation		1 sample	2 samples	3 samples
IBI score	15.0	3.87	**4.97**	3.51	2.87
Total Taxa Richness	7.61	2.76	3.54	2.50	2.04
EPT Richness (PTV 0 – 4)	2.48	1.57	2.02	1.43	1.17
Beck's Index, version 3	8.87	2.98	3.82	2.70	2.20
Shannon Diversity	0.04	0.20	0.26	0.18	0.15
Hilsenhoff Biotic Index	0.08	0.28	0.35	0.25	0.20
% Sensitive Individuals (PTV 0 – 3)	28.90	5.38	6.89	4.87	3.98

The information presented in Table 10 provides an estimate of the spatial precision of the IBI and each of the six core metrics. In other words, the lower the standard deviation, as estimated by the ANOVA MSE, the more confident we can be in methodological precision at a given site. These estimates of IBI and metric precision incorporate natural intrasite spatial variability and methodological variability.

Temporal Variability

Eighty-nine sites were sampled on more than one date, ranging from two to nine samples taken over time at a given site. Only one sample from each site was used in development of the IBI, but analysis of all samples from the same sites over time can provide an estimate of temporal variability of the index. Table 10 shows results from a one-way ANOVA with site as a factor performed on the samples from sites sampled at multiple times. Like the numbers in Table 10, these estimates of IBI and metric precision incorporate natural intrasite spatial variability and methodological variability, but they also incorporate natural temporal variability and variability due to changes in condition over time.

Table 10. Results of a one-way ANOVA with site as a factor performed on the samples from sites sampled at multiple times.

Metric	ANOVA MSE	Square Root of ANOVA MSE	One-tailed 90% Confidence Interval		
	estimates of within group standard deviation		1 sample	2 samples	3 samples
IBI score	72.1	8.49	**10.89**	7.70	6.28
Total Taxa Richness	18.90	4.35	5.57	3.94	3.22
EPT Richness (PTV 0 – 4)	7.29	2.70	3.46	2.45	2.00
Beck's Index, version 3	23.80	4.88	6.25	4.42	3.61
Shannon Diversity	0.08	0.29	0.37	0.26	0.21
Hilsenhoff Biotic Index	0.54	0.74	0.94	0.67	0.54
% Sensitive Individuals (PTV 0 – 3)	238.00	15.43	19.78	13.98	11.42

Application to an Independent Dataset

In an effort to further evaluate performance, the IBI was applied to 112 samples collected from wadeable freestone streams in Pennsylvania for a separate project (USEPA's REMAP) using the same methodology. All REMAP samples were collected between March 30, 2005 and May 27, 2005 by non-DEP biologists. None of these REMAP samples were used in the IBI development process. For purposes of comparison with the IBI development dataset, the abiotic condition tier assignment process described above was applied to the REMAP sites. The IBI distinguished very well between condition tiers as defined for this project using the REMAP samples (Figure 12).

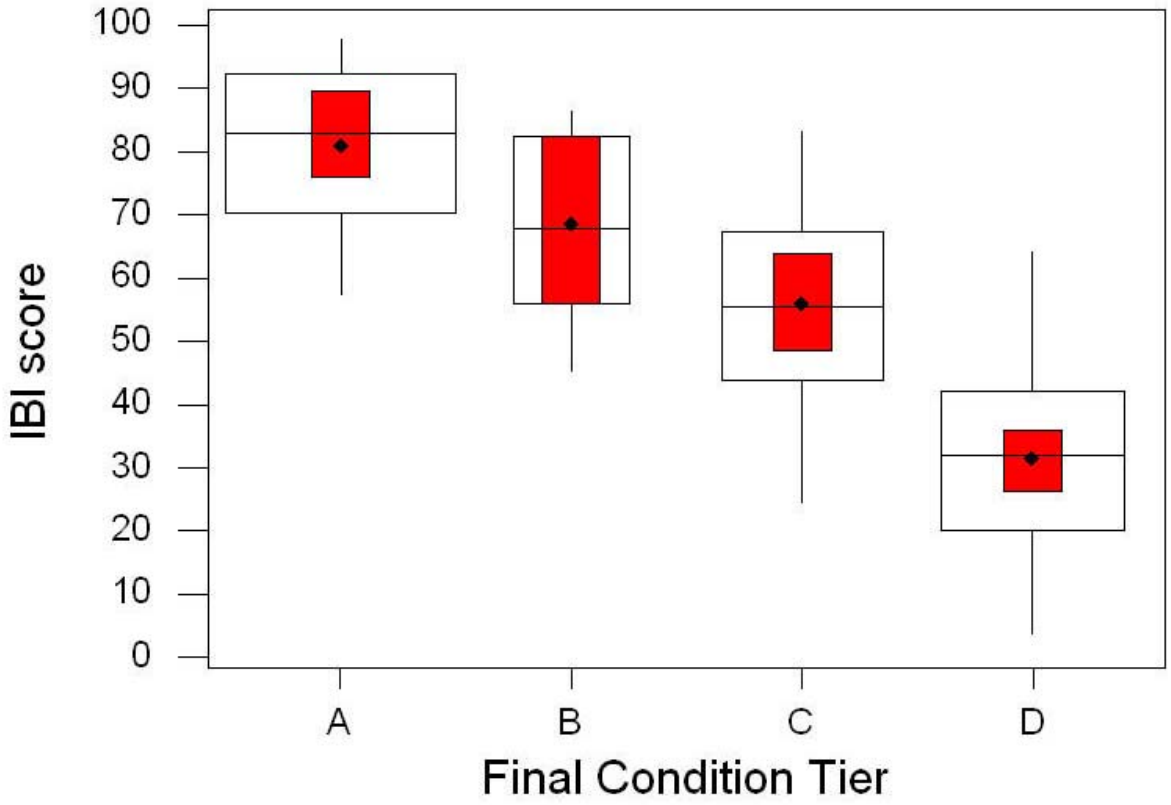

Figure 12. Boxplot of IBI scores for samples collected in Pennsylvania for the USEPA REMAP project.

During REMAP, duplicate biological samples were taken at four sites on the same day to provide further insight into intrasite spatial variability (Table 11).

Table 11. IBI scores for REMAP sites sampled multiple times on the same day.

Sample ID	Site Name	Site Condition Tier	IBI Score
20050527-1030-DRB	Bush Kill	A	79.0
20050527-1045-DRB			79.9
20050524-0930-DRB	McMichaels Creek	B	70.5
20050524-1000-DRB			70.5
20050524-1400-DRB	O'Donnels Creek	A	71.3
20050524-1415-DRB			76.0
20050512-1230-DRB	Sandy Run	C	46.7
20050512-1330-DRB			51.7

The variability of the IBI scores for each pair of replicate samples was very low (3.14 for a 1-sample 90% confidence interval – compare to Table 9 and Table 10 above).

PENNSYLVANIA TIERED AQUATIC LIFE USE WORKSHOPS

Numerous professional aquatic biologists gathered in Harrisburg, Pennsylvania on three separate occasions (August 8 and 9, 2006; August 22 and 23, 2007; May 15 and 16, 2008) to conduct tiered aquatic life use (TALU) workshops. The underlying concepts and procedural details of these workshops are described elsewhere (Gerritsen and Jessup 2007) and available on request, but the basic idea of the workshop was to assign benthic macroinvertebrate samples to one of a series of biological condition tiers. Good agreement among 45 biologists participating in the three TALU workshops and consistency with empirical evidence indicates the conceptual biological condition gradient (BCG) model reflects important aspects of biological condition along a general stressor gradient (Davies and Jackson 2006). Davies and Jackson (2006) promote use of the BCG as a descriptive model of ecosystem response to stress using six conceptual tiers (Figure 13).

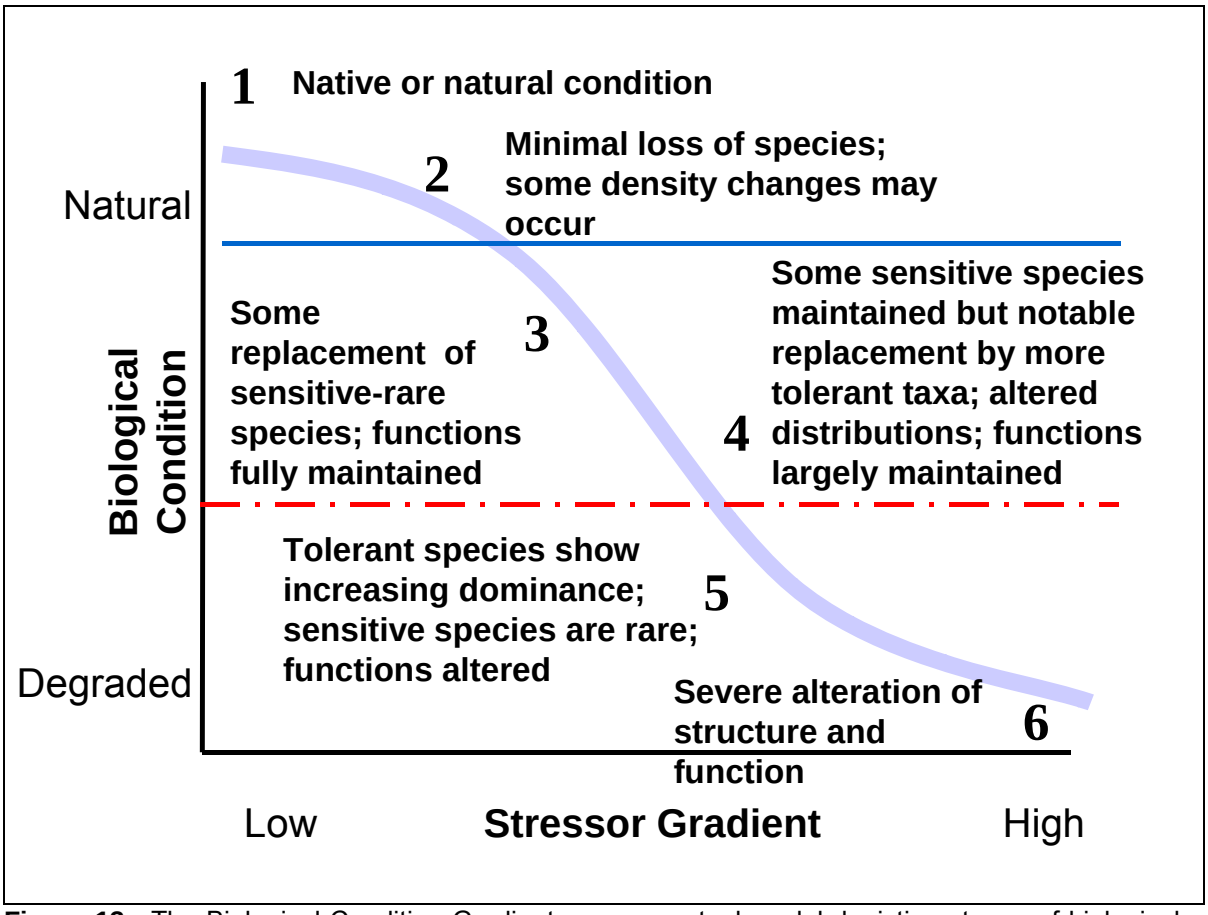

Figure 13. The Biological Condition Gradient – a conceptual model depicting stages of biological condition responses to an increasing stressor gradient – a*dapted from Davies and Jackson (2006)*.

Davies and Jackson (2006) offer that the biological condition required to support an ALU for a specific water body can be described in terms of BCG tiers. For example, the biological condition associated with wild brook trout reproduction requires a very high-quality stream and may be defined as a narrow range of nearly natural BCG tiers, while

the biological condition needed to support warm water recreational fisheries may span a broader range of conditions. Davies and Jackson (2006) note that individual applications of the BCG may not require – or be able to distinguish – six tiers, but the BCG development group concluded that six biological condition tiers can be qualitatively distinguished by well-designed and rigorous monitoring programs and that smaller increments of change are useful to show improvements or losses in biological condition.

In addition, the biologists who participated in development and testing of the BCG reported that the ecological characteristics conceptually described by tiers 1 through 4 correspond to how they interpret the CWA interim goal for protection and propagation of aquatic life (Davies and Jackson 2006). Further, the same biologists identified the characteristics described by tiers 1 and 2 as indicative of biological integrity (Davies and Jackson 2006).

Potential pitfalls of the BCG approach include: (1) lack of assessment experience and difficulty of practically and accurately assessing the status of some BCG attributes (e.g., ecosystem function); (2) a consensus definition of tier 1 conditions; and (3) the lack of regionally evaluated species tolerance to general and specific stressors.

The results of the Pennsylvania TALU workshops indicate that professional aquatic biologists from a number of organizations with extensive experience sampling benthic macroinvertebrates and other aquatic life (e.g., fish, periphyton) in the region generally agree on the characteristics exhibited by “reference condition” or “natural” benthic macroinvertebrate communities in the Commonwealth for these types of streams. This is an important finding that provides consistent meaning to quantification of these characteristics and decisions based on biological criteria for ALU attainment. Generally, IBI scores and BCG tier assignments for the 105 samples evaluated at the three TALU workshops agreed very well (Figure 14 – 17), however, some IBI scores fell a bit short of TALU tier assignments for larger streams rated BCG tier 3 or higher (Figure 16). It should be noted that the IBI scores presented by Gerritsen and Jessup (2007) are based on a different set of metrics than the IBI developed in this report.

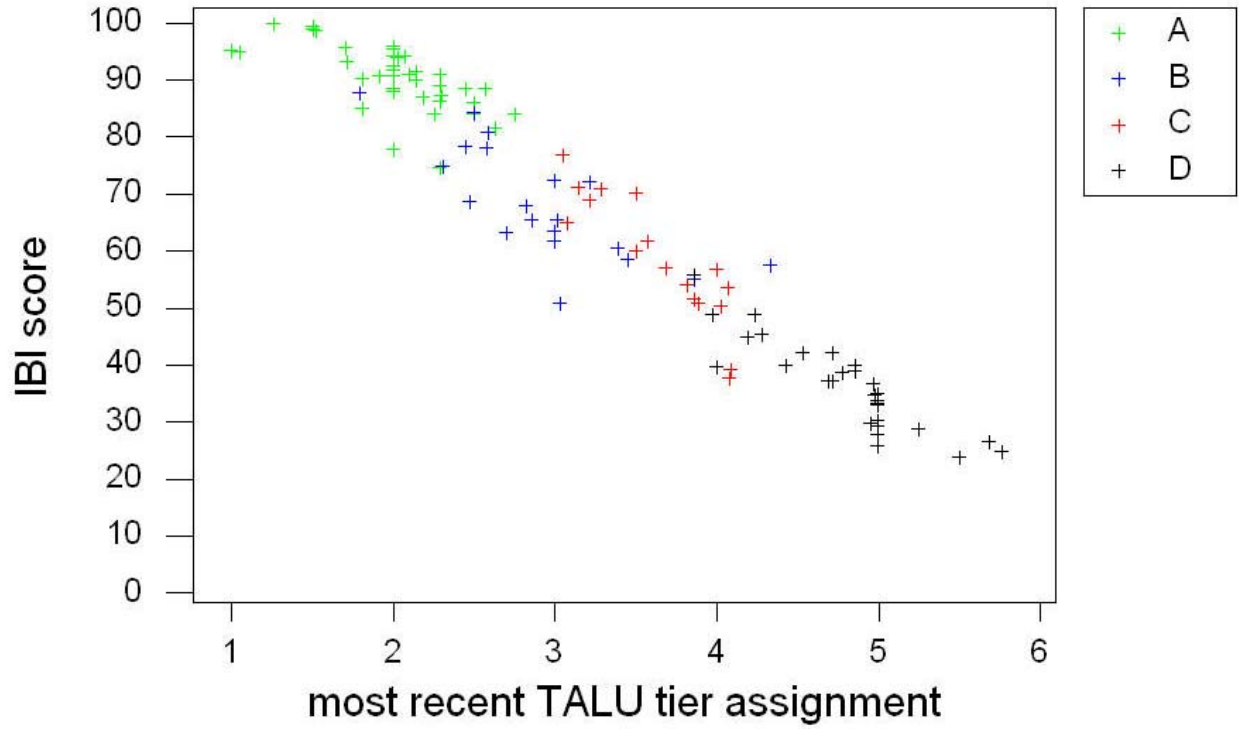

Figure 14. IBI scores for 105 samples plotted against the average assigned at the most recent TALU workshop tier and coded according to final sample condition type (r^2 = 0.94 for a linear regression of the IBI score and most recent TALU tier assignment).

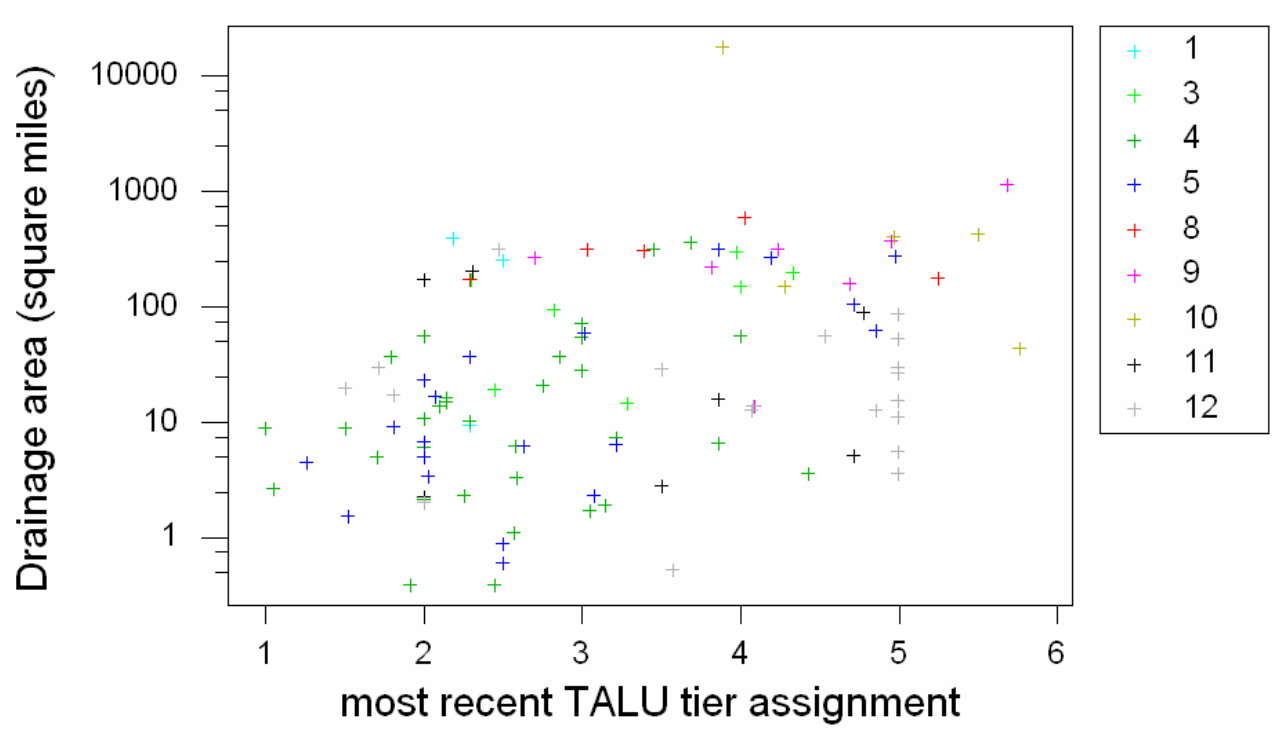

Figure 15. Drainage area for 105 samples plotted against the average assigned at the most recent TALU workshop tier and coded according to sample month.

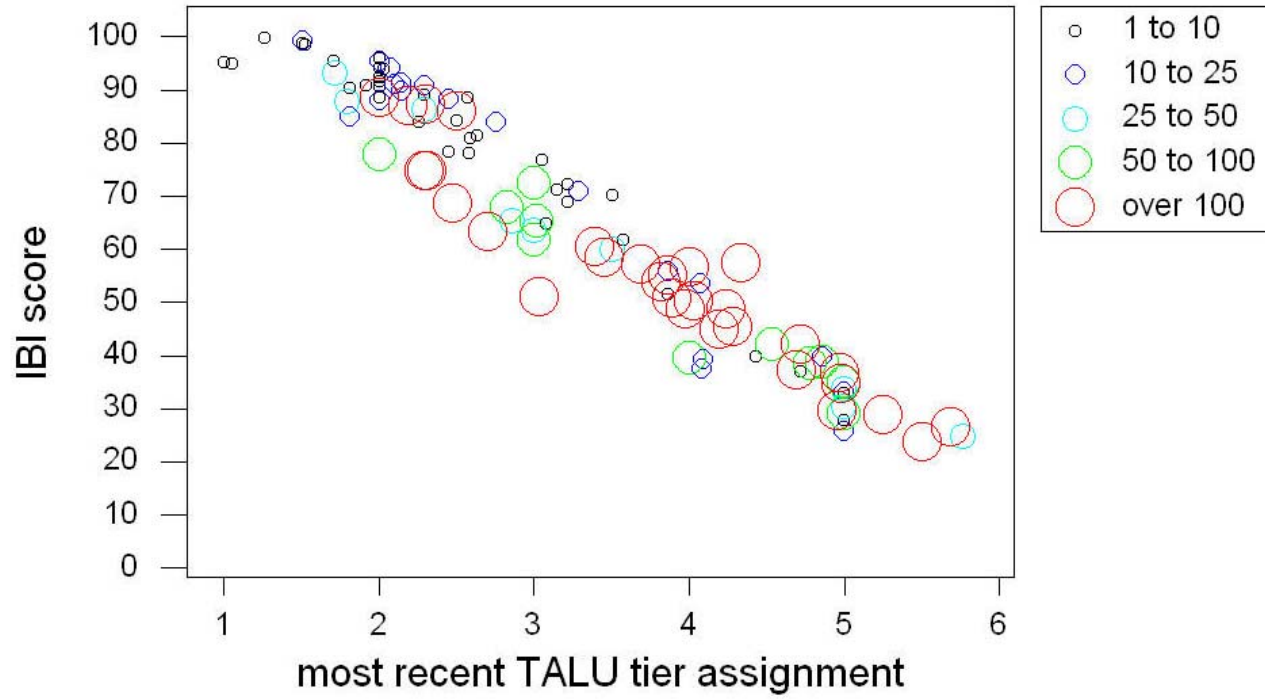

Figure 16. IBI scores for 105 samples plotted against the average assigned at the most recent TALU workshop tier and coded according to drainage area.

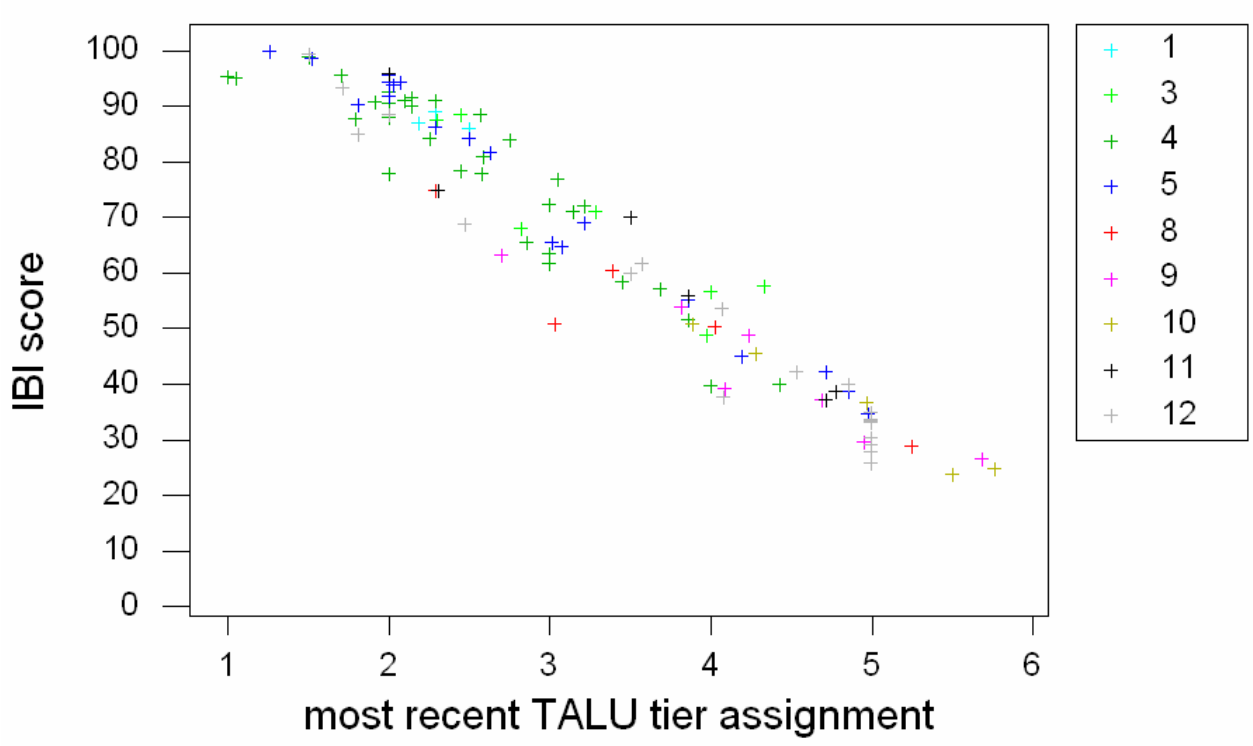

Figure 17. IBI scores for 105 samples plotted against the average assigned at the most recent TALU workshop tier and coded according to sample month.

AQUATIC LIFE USE ATTAINMENT BENCHMARKS

For purposes of assessing ALU attainment based on IBI scores, use attainment thresholds or benchmarks can be established for specific stream types, regions and ALU levels. The multimetric index approach offers the ability to use a single index score to simplify management and decision-making (Barbour et al. 1999). The single index value may not determine the exact nature of stressors affecting the ecosystem, but analysis of the individual metrics may offer some insight into causes of ecosystem stress (Barbour et al. 1999). Thus, the index score can be used as a stand-alone assessment tool to represent aquatic life use attainment status, but the assessment process may be strengthened by considering the index score in concert with other available information (Barbour et al. 1999).

A number of somewhat subjective decisions are involved in establishment of any use attainment thresholds or categorization of biological condition as acceptable or unacceptable. The selection of the appropriate criteria heavily depends on the nature of the samples in the dataset, especially the samples used to define the reference condition (Hughes 1995; Barbour et al. 1999; Stoddard et al. 2006), which is why the site condition tier definition process was carefully and thoroughly considered for this project. The extremes of biological condition (i.e., severely degraded and nearly pristine conditions) are usually easier to deem acceptable or unacceptable deviations from natural conditions than middle-of-the-road conditions (Hughes 1995). Any set of undisturbed sites will naturally exhibit a range of scores at any point in time (Stoddard et al. 2006), which is why spatial and temporal precision of the index were estimated for this project. Barbour et al. (1999) recommend using established percentiles of multimetric index scores for the reference sites to discriminate between severely degraded and nearly natural conditions. Barbour et al. (1999) also note that the range of index scores can be subdivided into any number of categories corresponding to various levels of degradation or use attainment.

Based on the results of the classification analyses discussed above, DEP decided not to establish separate reference conditions and thresholds for wadeable freestone, riffle-run type streams in separate regions of the Commonwealth. However, due to the influences of annual seasons and drainage area seen in the dataset, DEP recognizes different use attainment thresholds may be appropriate for samples collected during different times of the year and from different size stream systems. It should be re-emphasized that the index development dataset was mostly comprised of samples from relatively small wadeable freestone, riffle-run type streams. It is noted that some site-specific exceptions to any thresholds may exist because of local scale natural limitations (e.g., habitat availability) on biological condition (Hughes 1995).

Based on the results of the analyses presented above, the results of the TALU workshops and feedback from DEP biologists and policy considerations, DEP implements a multi-tiered benchmark decision process for smaller wadeable freestone riffle-run streams in Pennsylvania that incorporates sampling season as a factor for

determining ALU attainment and impairment for the CWF, WWF and TSF protected uses (Figure 20).

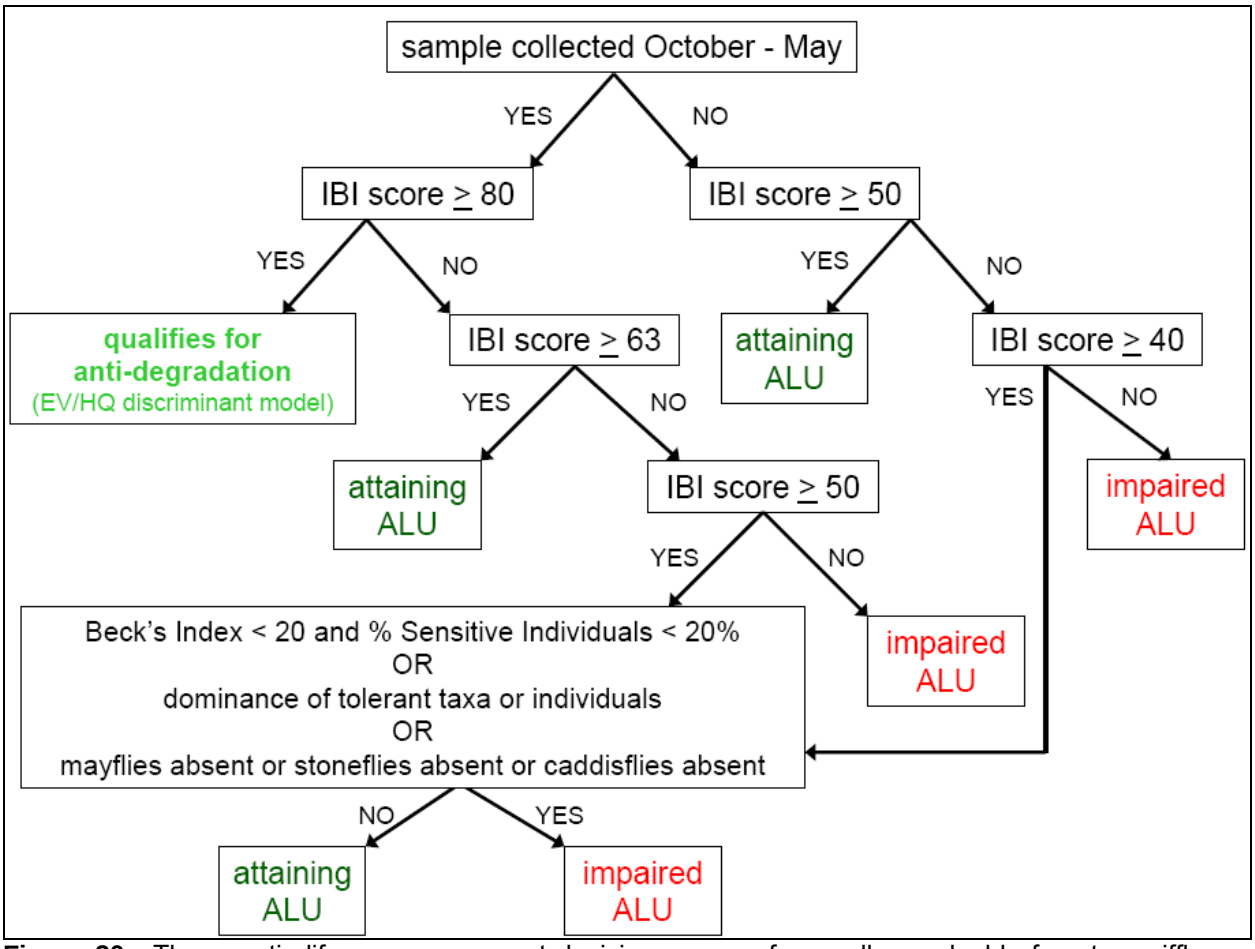

Figure 20. The aquatic life use assessment decision process for smaller wadeable freestone riffle-run type streams in Pennsylvania.

The first step in the ALU assessment process for smaller wadeable freestone streams in Pennsylvania considers sampling season (i.e. June through September versus October through May) since the analyses presented above and in Appendix E show that certain metric scores tend to drop during the summer months. These seasonal index periods are intended as general guidelines and may vary slightly year-to-year depending on climatological conditions; for example, a sample collected during the last week of May in a particularly hot, dry year may be more properly evaluated using procedures set forth for the summer months.

The IBI anti-degradation benchmark for smaller streams (i.e., IBI score ≥ 80) is only applicable from October through May; samples for anti-degradation surveys in wadeable freestone streams should not be collected during the summer months (i.e., June to September). Samples that qualify for anti-degradation consideration are subject to a discriminant model that then determines their status as either exceptional value (EV) or high quality (HQ) anti-degradation tiers (Pennsylvania Department of

Environmental Protection 2008). The antidegradation candidacy benchmark scores will be implemented as qualifiers for special protection status only (i.e., a sample with an IBI score at or above the applicable benchmark will be afforded special protection status); they will not be used as impairment thresholds for special protection waters. The majority of the original EV and HQ designations were not based on biological sampling. As a result, we do not know what IBI score these streams may have had when and since they were designated special protection. Due to this uncertainty, we will protect a special protection stream at the IBI measured during a current survey minus the temporal precision estimated for the IBI development dataset (11.0 IBI points). For example, if Pine Run is designated HQ with no available historic metric values or IBI scores and the new method results in an IBI score of 75.0, the stream will retain its HQ status and IBI scores from future surveys will not be allowed to fall below 64.0 (75.0 minus 11.0) without listing the stream as impaired. Special protection sites that generate relatively low IBI scores will be protected at the 63.0 impairment benchmark. For example, if Mud Run scores 68.0, the IBI will not be allowed to fall below the 63.0 impairment threshold; the impairment threshold would not be lowered to 57.0 (68.0 minus 11.0). There will be exceptional circumstances when the above scoring thresholds do not apply (e.g., when there are obvious sources of impairment or the stream never deserved special protection status); however, for the majority of cases, the IBI scoring and use attainment thresholds described in the previous paragraph will apply.

For samples collected from smaller streams between October and May, an IBI score $\geq$ 63 results in ALU attainment and an IBI score < 50 results in ALU impairment; an IBI score between 50 and 63 requires further evaluation to determine ALU impairment – three guidelines may be used: (1) if the Beck's Index score is < 20 and the % Sensitive Individuals in the sub-sample is < 20%, the ALU should be impaired without compelling reason otherwise; (2) if the sample is dominated by tolerant taxa or individuals, the ALU should be impaired without compelling reason otherwise; or (3) if mayflies, stoneflies or caddisflies are absent from the sub-sample the ALU should be impaired without compelling reason otherwise.

For samples collected between June and September from smaller streams, an IBI score $\geq$ 50 results in ALU attainment and an IBI score < 40 results in ALU impairment; an IBI score between 40 and 50 requires further evaluation to determine ALU impairment, guided by the same three guidelines outlined above for October to May samples scoring between 50 and 63 (although the absence of mayflies in samples collected immediately after spring hatches may be relaxed in some cases). The basis for lowering the impairment threshold range to an IBI range of 40 to 50 in the summer months is that all tier A sites score above 50, even in the summer months, while some tier B sites dip below 50, but not 40, in the summer months (Figure 21). In addition, there were a number of examples of sites with IBI scores above 50 from October to May that scored in the 40 to 50 range during the summer months (Figure 22). The seasonality of IBI metric scores is presented graphically in Appendix E.

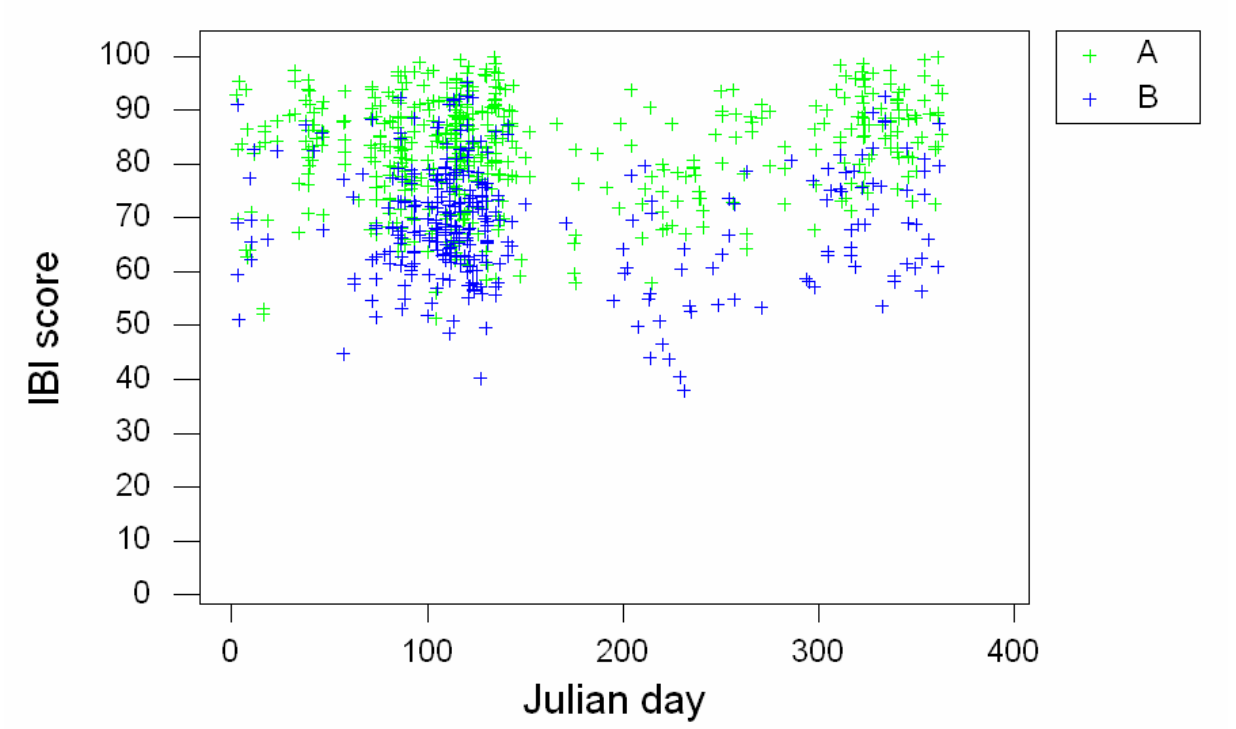

Figure 21. IBI scores versus Julian day for all tier A and B samples.

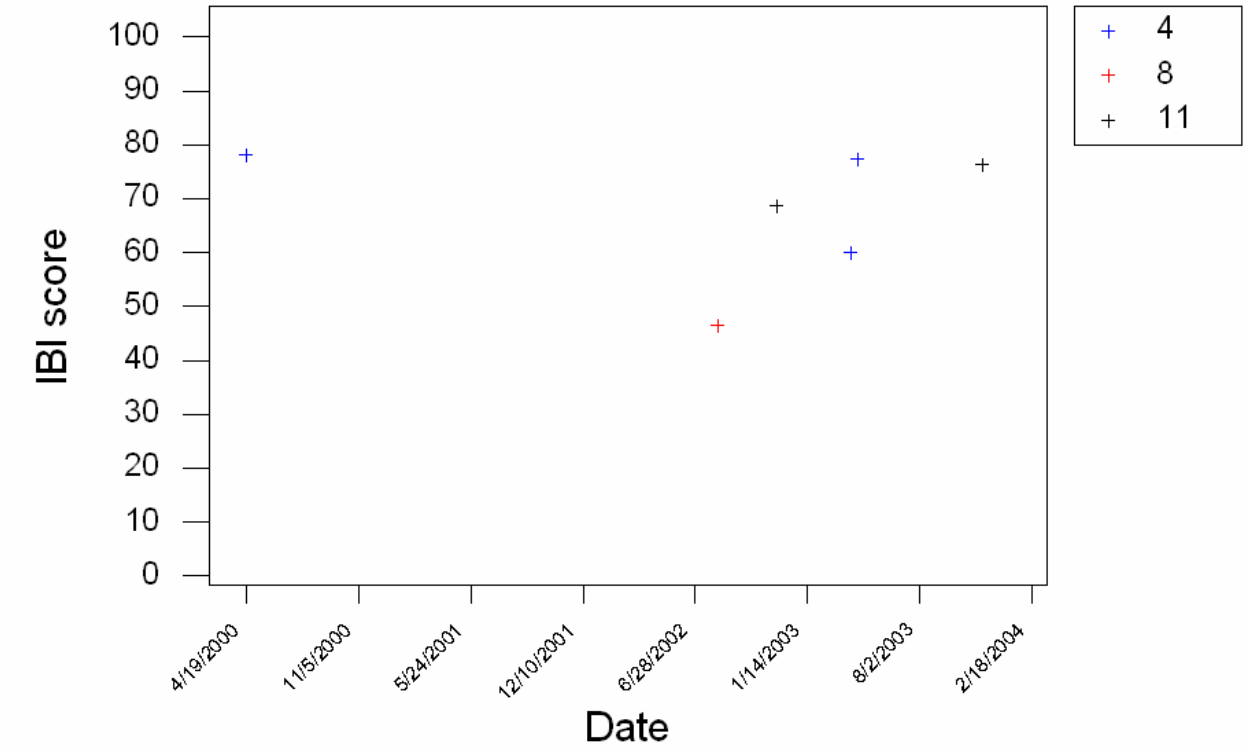

Figure 22. IBI scores versus sampling date for six samples from Choke Creek in Luzerne/Lackawanna County – a tier B site draining 1.8 square miles – color coded by sample month. Note that the April and November samples score above 50 while the August sample scores in the 40 to 50 range. This site is just one example of a number of other sites with similar seasonal IBI score patterns.

For larger wadeable freestone riffle-run type streams, DEP believes more samples are necessary to accurately establish ALU attainment and impairment benchmarks. Given the nature of flowing water bodies as gradually changing continuums, it is difficult to define a specific numeric cutoff to separate larger streams from smaller streams. However, the present dataset suggest that scores for some index metrics begin to decline for reference-quality streams drainage areas reach the 25 to 50 square mile range (see Appendix E). The 2008 TALU workshop, which dealt mostly with samples from larger freestone stream systems, confirms that biological expectations or potential for most of the relatively pristine larger freestone streams in Pennsylvania are less than the biological expectations or potential for the relatively pristine smaller freestone streams (Figure 16). The reasons for the decreased metric and index expectations in relatively pristine larger stream systems are primarily attributable to natural changes in thermal regime, trophic dynamics and habitat types as a stream progresses from its headwaters downstream to its mouth (Vannotte et al. 1980), which result in natural shifts in the benthic macroinvertebrate community away from taxa that thrive in colder water and are adapted to shredding leaf litter (e.g., many stonefly genera) toward taxa that thrive in warmer water and are adapted to collecting or filtering fine particulate organic matter (e.g. some Hydropsychiidae caddisfly genera) or scraping periphyton from substrate (e.g., Isonychiidae mayflies).

For samples collected from smaller freestone streams between October and May, the established ALU special protection benchmark (i.e., IBI score $\geq$ 80) corresponds to a TALU tier of approximately 2.5 and includes all tier A samples draining less than 50 square miles (Figure 16, Figure 23) and the established ALU attainment (i.e. IBI score $\geq$ 63) and impairment (i.e., IBI score < 50) benchmarks correspond to TALU tiers of approximately 3.0 and 4.0, respectively (Figure 23).

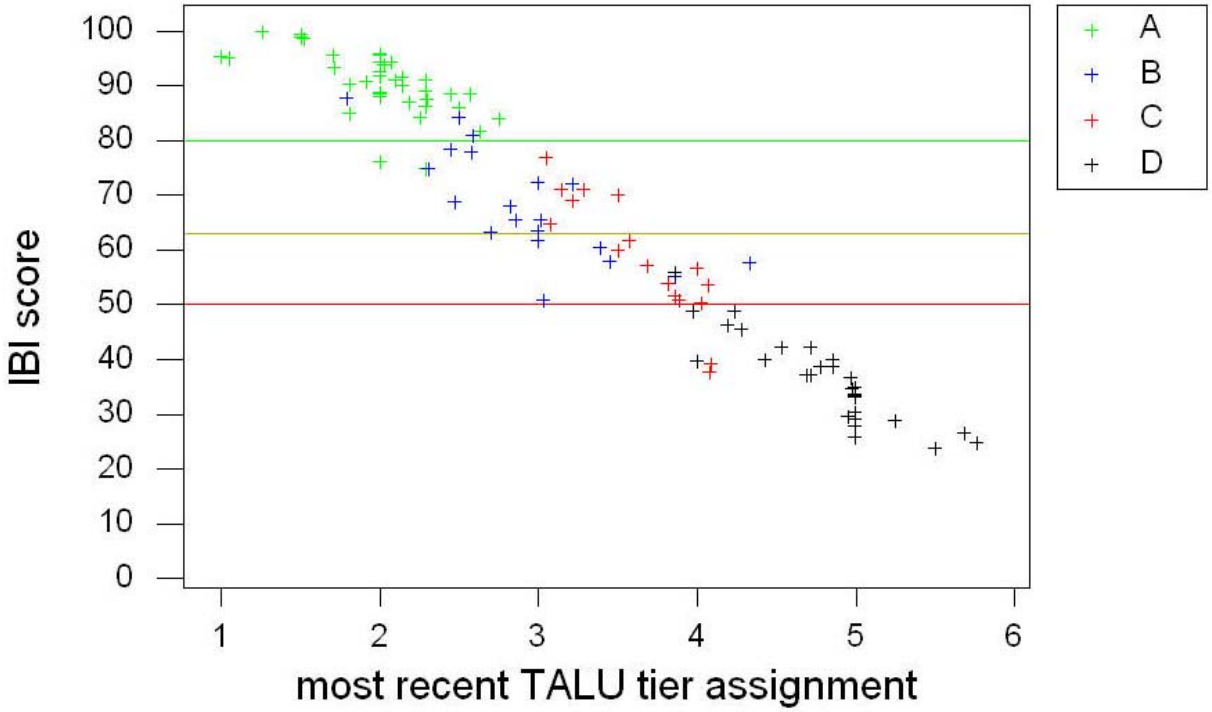

Figure 23. IBI scores for 105 samples plotted against the average assigned at the most recent TALU workshop tier and coded according to final sample condition type. Established IBI score aquatic life use benchmarks for samples collected between October and May are shown for reference: special protection qualification ≥ 80; use attainment ≥ 63; use impairment < 50.

The use assessment decision process and accompanying attainment/impairment benchmarks set forth above are intended as general guidelines, not as hard-and-fast rules. While the above guidelines will provide an accurate assessment of benthic macroinvertebrate community condition for the vast majority of samples collected from wadeable, freestone, riffle-run streams in Pennsylvania, there will be instances where a biologist's local knowledge of conditions may warrant a decision not arrived at using these guidelines. For instance, if a sample is heavily dominated by Simuliidae or Chironomidae larvae, often times this will make the metric and IBI scores difficult to interpret and the investigating biologist must rely on a more qualitative analysis of the metric scores and sample composition to arrive at an assessment decision. Similarly, samples from streams in areas receiving a substantial amount of flow from groundwater attributable to limestone geology are naturally expected to have less diversity than "true freestone" streams, so use attainment benchmarks may be justifiably relaxed for samples from these types of streams.

Multi-tiered management approaches are useful because they can help to: (1) identify the highest quality resources; (2) describe a gradient of biological conditions; (3) set realistic and attainable goals; (4) document and help preserve incremental improvements; and (5) trigger action when conditions deteriorate (see Davies and Jackson 2006).

DEP's current antidegradation requirements consider both water chemistry and biology for qualification as HQ. Specific chemical requirements are outlined in Chapter 93.4b.(a)(1). Biological requirements in Chapter 93.4b.(a)(2) are summarized below and provide that a surface water can qualify as HQ if one or more of the following conditions exist:

- the surface water supports a high quality aquatic community based upon information gathered using widely accepted and published peer-reviewed biological assessment procedures that DEP may apply or approve to determine the condition of the aquatic community of a surface water
- the surface water has been designated a Class A wild trout stream by the Pennsylvania Fish and Boat Commission following public notice and comment

The antidegradation requirements further state in Chapter 93.4b.(b) that a water qualifies as EV if it is a surface water of exceptional ecological significance or if it qualifies as an HQ water and meets one or more of the following conditions:

- the water is located in a national wildlife refuge or state game propagation and protection area
- the water is located in a designated state park natural area or state forest natural area, national natural landmark, federal or state wild river, federal wilderness area, or national recreational area
- the water is an outstanding national, state, regional, or local resource water
- the water is a surface water of exceptional recreational significance
- the water is designated as a "wilderness trout stream" by the Pennsylvania Fish and Boat Commission following public notice and comment

Chapter 93.4b. also states that surface waters may be considered as antidegradation waters by site-specific comparison to an integrated benthic macroinvertebrate score of a reference stream or watershed (i.e., scoring 83% of reference for HQ, scoring 92% of reference for EV); the population-based IBI approach outlined in this project will replace this site-specific reference comparison approach (i.e., currently outlined in Chapter 93). As stated above, with the population-based IBI approach, antidegradation candidacy requires a minimum IBI score of 80.0 for smaller streams. Further distinction between EV and HQ designations will primarily rely on a statistical discriminant model developed by DEP (Pennsylvania Department of Environmental Protection 2008), while also drawing on the points listed above and determinations of exceptional ecological significance as described in guidance.

FUTURE DEVELOPMENT

The index developed in this project is based solely on the benthic macroinvertebrate assemblage. The index primarily measures structure of that assemblage, not function or measures of individual condition. Sampling of multiple assemblages (e.g., fish, periphyton) may provide improved stressor detection capability over a broader range of conditions. However, for purposes of monitoring and assessment of stream ecosystems, DEP believes the structure of benthic macroinvertebrate communities viewed at the described taxonomic resolution represents an informative, cost-effective indicator that is sensitive to a wide range of stressors of water quality in Pennsylvania streams. Any management decisions should evaluate all pertinent, available data, not just rely on this index if other information is available to help assess water quality.

The geographic range of samples in the IBI development dataset represented many parts of the Commonwealth, but this dataset was more heavily concentrated in certain watersheds than others owing to DEP's current sampling strategy, which rotates focus from watershed to watershed (defined as 8-digit hydrologic unit codes by the United States Geological Survey) each year. For example, sampling density was much higher in the Middle Allegheny-Tionesta, Youghiogheny, Monocacy, Lower West Branch Susquehanna, Schuylkill, Brandywine-Christina and Lehigh watersheds. As DEP's sampling strategy moves from watershed to watershed, more samples will be collected from other areas of the state and this project should be revisited periodically as data are collected from more streams in Pennsylvania. While revisiting this project, an effort should be made to: (1) collect habitat and water quality data for all newly sampled sites and for existing sites where such data is currently not available; (2) incorporate additional data not considered in this analysis (e.g., number of upstream road crossings); and (3) refine and update existing data (e.g., slope measurements, land use calculations). Some of the reference sites used in development of this IBI should be re-sampled repeatedly to document reference condition variation over time. The need to revise the current seasonal classification system, or to implement a regional classification system, may become apparent and there may be a need to revise metric selection, index development or use attainment thresholds as more samples are collected and analyzed. During refinement of the IBI, special attention should be given to appraisal of the importance of drainage area and stream gradient, as both parameters exerted a noticeable influence on benthic macroinvertebrate communities and DEP already recognizes the importance of stream gradient through its separate multihabitat sampling protocol for low-gradient, pool-glide type streams. In addition, factors such as elevation, latitude and alkalinity should continue to be carefully evaluated as the project is refined.

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Appendix A – Field Sampling and Lab Methods

1. Habitat Assessments

The Department has adopted the habitat assessment methods outlined in USEPA's Rapid Bioassessment Protocols (RBP; Plafkin, et al. 1989) and subsequently modified[1]. The matrix used to assess habitat quality is based on key physical characteristics of the water body and surrounding lands. All parameters evaluated represent potential limitations to the quality and quantity of instream habitat available to aquatic biota. These, in turn, affect community structure and composition.

The main purpose of the habitat assessment is to account for the limitations that are due to existing stream conditions. This is particularly important in cause/effect and cumulative impact studies where the benthic community at any given station may already be self-limited by background watershed and habitat conditions or impacts from current land uses. In order to minimize the effects of habitat variability, every effort is made to sample similar habitats at all stations. The habitat assessment process involves rating twelve[1] parameters as excellent, good, fair, or poor, by assigning a numeric value (ranging from 20 - 0[1]), based on the criteria included on the Habitat Assessment Field Data Sheets (Riffle/Run and Glide/Pool, Appendix A).

The twelve habitat assessment parameters used in the DEP-RBP evaluations for Riffle/Run prevalent (and Glide/Pool prevalent) streams are discussed below. The Glide/Pool parameters that differ from the Riffle/Run parameters are shown in italics. The first four parameters evaluate stream conditions in the immediate vicinity of the benthic macroinvertebrate sampling point:

- **Instream Fish Cover** - evaluates the percent makeup of the substrate (boulders, cobble, other rock material) and submerged objects (logs, undercut banks) that provide refuge for fish.

- **Epifaunal Substrate** - evaluates riffle quality, i.e. areal extent relative to stream width and dominant substrate materials that are present. *(In the absence of well-defined riffles, this parameter evaluates whatever substrate is available for aquatic invertebrate colonization.)*

- **Embeddedness** - estimates the percent (vertical depth) of the substrate interstitial spaces filled with fine sediments. ***(pool substrate characterization:*** *evaluates the dominant type of substrate materials, i.e. gravel, mud, root mats, etc. that are more commonly found in glide/pool habitats.)*

1. Plafkin et al. originally presented nine habitat assessment parameters divided into three different scoring ranges of 20-0, 15-0, and 10-0. Modifications to these original habitat methods were presented at several seminars following this 1989 publication. These modifications added one more habitat parameter to each of the three original categories; bringing the total parameters to 12. The scoring ranges eventually were increased to 20-0 for all 12. This Habitat Protocol has undergone several more iterations – resulting in yet more variations from the original and the Department's current 12 criteria - 20 point scoring habitat assessment method.

- **Velocity/Depth Regime** - evaluates the presence/absence of four velocity/depth regimes - fast-deep, fast-shallow, slow-deep, and slow-shallow. (Generally, shallow is <0.5m and slow is <0.3m/sec. ***Pool variability:*** *describes the presence and dominance of several pool depth regimes.*)

The next four parameters evaluate a larger area surrounding the sampled riffle. As a rule of thumb, this expanded area is the stream length defined by how far upstream and downstream the investigator can see from the sample point.

- **Channel Alteration** - primarily evaluates the extent of channelization or dredging but can include any other forms of channel disruptions that would be detrimental to the habitat.

- **Sediment Deposition** - estimates the extent of sediment effects in the formation of islands, point bars, and pool deposition.

- **Riffle Frequency (pool/riffle or run/bend ratio)** - estimates the frequency of riffle occurrence based on stream width. (***Channel sinuosity:*** *the degree of sinuosity to total length of the study segment.)*

- **Channel Flow Status** - estimates the areal extent of exposed substrates due to water level or flow conditions.

The next four parameters evaluate an even greater area. This area is usually defined as the length of stream that was electro-shocked for fish (or an approximate 100 meter stream reach when no fish were sampled). It can also take into consideration upstream land-use activities in the watershed:

- **Condition of Banks** - evaluates the extent of bank failure or signs of erosion.

- **Bank Vegetative Protection** - estimates the extent of stream bank that is covered by plant growth providing stability through well-developed root systems.

- **Grazing or Other Disruptive Pressures** - evaluates disruptions to surrounding land vegetation due to common human activities, such as crop harvesting, lawn care, excavations, fill, construction projects, and other intrusive activities.

- **Riparian Vegetative Zone Width** - estimates the width of protective buffer strips or riparian zones. This is a rating of the buffer strip with the least width.

It is best to conduct the habitat assessment after sampling since the investigator has observed all conditions in the sampled segment and immediate surrounding watershed. After all parameters in the matrix are evaluated and scored, the scores are summed to derive a habitat score for that station. The “optimal” category scores range from 240-192; “sub-optimal” from 180-132; “marginal” from 120-72; and “poor” is 60 or less. The gaps between these categories are left to the discretion of the investigator’s best professional judgment.

2. Benthic Macroinvertebrates

2.A. Net Mesh Considerations

In recent years, many state water quality programs, federal agencies (e.g. USEPA, USGS), and other water quality monitoring organizations began using net sampling devices with 500µ mesh nets. In order to conform to this trend, the 500µ net mesh size has been adopted for the Department's D-frame sampler used in the DEP-RBP sampling method (described below). Future references to the D-frame sampler in the document assume 500-µ mesh netting. The net mesh size of other screen samplers has not changed and still is to be 800-900 µ. Because of this net mesh size change, the mesh size of the sampler used must be noted on field and bench identification sheets for the collected benthic sample.

2.B. Qualitative Methods

The type of sampling gear used is dependent on survey type and site-specific conditions. The recommended gear in wadeable streams are 3' x 3' flexible kick-screens and 12-inch diameter round D-frame nets. In larger streams or rivers, grab-type samplers may be used to obtain qualitative samples. While generally thought of as quantitative devices, Eckman, Peterson, or Petite Ponar grab samplers can also be used to obtain qualitative data. The type of gear, dimensions, and mesh size must be reported for all collections. When more than one gear type is used, the results must be recorded separately.

Physical variables should be matched as closely as possible between background and impact stations when selecting locations for placement of the sampling gear within each station. Matching these variables helps minimize or eliminate the effects of compounding variables.

Macrobenthos often exhibit clustered distributions, and if the sampling points are selected in close proximity to each other, a single clustered population may be obtained rather than a generalized measure of the overall population within the selected sub-habitat. Spacing the sampling points as far apart as possible within the sub-habitat can minimize the problem of clustered distributions.

2.B.1. Kick-screen. A common qualitative sampling method uses a simple hand-held kick-screen. This device is designed to be used by two persons. However, with experience, it may be used by one person and still provide adequate results. The kick-screen is constructed with a 3' x 3' piece of net material (800-900 µ mesh size) fastened to two dowel handles (approximately 1"d. X 4' long).

2.B.1.a. Traditional Method. Facing up stream, one person places the net in the stream with the bottom edge of the net held firmly against the streambed. An assistant then vigorously kicks the substrate within a 3' x 3' area immediately upstream of the net to a depth of 3" - 4" (approximately 10 cm). The functional depth sampled may vary due to ease of disturbance as influenced by substrate embeddedness.

The amount of effort expended in collecting each sample should be approximately equivalent in order to make valid comparisons. The effort, expressed as area, must be reported for all collections.

Collect a minimum of four screens at each site. Initial sampling should be conducted in riffle areas. Collection in additional habitats to generate a more complete taxa list can be conducted at the discretion of the investigator. Initial analysis of the data must be limited to the riffle data for standardization. A second analysis including other habitats may be conducted as needed.

Data observations shall be recorded on a standard field sheet created for each station sampled. Record the relative abundance of each recognizable Family in each individual collection in the field. Relative abundance categories, with the observed "total" ranges indicated in parenthesis include: rare (0-3), present (3-10), common (11-24), abundant (25-99), and (occasionally) very abundant (100+). The investigator, at his/her discretion, may elect to enumerate certain target taxa.

Recording the results of each collection has several advantages that are lost if the data are composited for each station:

a. A stressed or enriched community often exhibits little variability in community structure over an area while a healthy community should have a more complex structure. If varied taxa are found on each screen, the community is probably complex, while the presence of only a few dominant taxa on every screen indicates the community is a simple one.

b. Collecting intolerant taxa in a majority of screens is a good indication of an unstressed community. However, collecting intolerant taxa in only one out of four screens may be an indication that the intolerant taxa have only a marginal existence at that location. A comparison of the composited taxa lists for each location may not indicate the rarity of the intolerant taxa, but this rarity would be readily apparent if the taxa lists for individual screens were compared.

c. Separate screen taxa lists provide information concerning the distribution of taxa. For example, mayflies are taken in one of four screens at the background station and in none of the four screens at the impact station. All the other taxa collected at both the stations are tolerant forms. Based on a composited taxa list for each station, one might conclude that the impact station is depressed due to the absence of mayflies. However, the individual screen taxa lists would indicate that the mayflies may have a clumped distribution and there is a possibility that the collector simply missed the clumps at the impact station. This will be apparent to the biologist while in the field and he/she can continue collecting until comfortable that mayflies are indeed absent or less abundant at the impact station. Later, it can be reported, for example, that 4 of 10 screens contained mayflies at the background station while only 1 of 10 screens contained mayflies at the impact station. This is an instance when the collector, while still in the field, may choose to count the mayflies in each screen (especially if the background screens had many mayflies while the impact screens only had one or two).

d. Separate screen data can lend weight to an analysis when classification techniques (ordination or clustering) are used. Results that cluster or score the individual background screens differently than the individual impact screens indicates a difference between the locations. When the classification technique scores background and impact screens in an apparent random manner, then it is likely that there is no impact or that the natural variability is large and masks any impacts.

Individuals of representative taxa for a station may be composited in a single vial and preserved for later laboratory verification or identification. Generally, the level of taxonomic identification would follow that as listed in section 2.E.1.

Answers to several questions can be useful in subsequent analysis and can be stored with the taxa lists as remark fields. The answers to the following questions, which require collector judgment, can be recorded in the field on a coded form. What are the dominant and rare taxa? Are there any taxa that are found to be unusually abundant?

2.B.1.b Assessment Method. This method is used for assessments conducted as part of the Statewide Surface Waters Assessment Program and employs the same kick screen gear, physical disturbance techniques, and relative abundance determinations as the traditional method (2.B.1.a). The main difference is that only two kicks are usually required and macroinvertebrate identifications are done streamside to family level taxonomy with hand-held lens (10X) if necessary. Data are recorded on standard field forms. Refer to the Statewide Surface Waters Assessment Protocol for further details.

2.B.2. D-Frame. The handheld D-frame sampler consists of a bag net attached to a half-circle ("D" shaped) frame that is 1' wide. The net's design is that of an extended, round bottomed bag (500µ mesh size). The methodology is basically the same as with the kick-screen - except for the following points: one person, facing downstream and holding the net firmly on the stream bottom, employs the net. One "**D-frame effort**" is defined as such: the investigator vigorously kicks an approximate area of 1 m^2 immediately upstream of the net to a depth of 10 cm (or approximately 4", as the embeddedness of the substrate will allow) for approximately one minute. All benthic dislodgement and substrate scrubbing should be done by kicks only. Substrate handling should be limited to only moving large rocks or debris (as needed) with no hand washing. Since the width of the kick area is wider than the net opening, net placement is critical in order to assure all kicked material flows toward the net. Avoiding areas with crosscurrents, the substrate material from within the square meter area should be kicked toward the center of the area – above the net opening.

The concepts and field forms concerning field recording of invertebrate data discussed in the kick-screen method section (2.B.1a) also apply to the D-frame method.

2.C. Semi-Quantitative Method (DEP-RBP):

In Plafkin (1989), USEPA presented field-sampling methods designed to assess impacts normally associated with pollution impacts, cause/effect issues, and other water quality degradation problems in a relatively rapid manner. These are referred to as Rapid Bioassessment Protocols (RBPs). The DEP-RBP method is a bioassessment

technique involving systematic field collection and subsequent lab analysis to allow detection of benthic community differences between reference (or control) waters and waters under evaluation. The DEP-RBP is a modification of the USEPA RBP III (Plafkin, et al; 1989); designed to be compatible with Pennsylvania's historical database. Modifications include: 1) the use of a D-frame net for the collection of the riffle/run samples, 2) different laboratory sorting procedures, 3) elimination of the CPOM (coarse particulate organic matter) sampling, and 4) metrics substitutions. Unlike the USEPA's RBP III methodology, no field sorting is done. Only larger rocks, detritus, and other debris are rinsed and removed while in the field before the sample is preserved. While USEPA's RBP III method was designed to compare impacted waters to reference conditions (cause/effect approach), the DEP-RBP modifications were designed for un-impacted waters, as well as impacted waters.

2.C.1. Sample Collection. The purpose of the standardized DEP-RBP collection procedure is to obtain representative macroinvertebrate fauna samples from comparable stations. The DEP-RBP assumes the riffle/run habitat to be the most productive habitat. Riffle/run habitats are sampled using the D-frame net method described above. The number of D-frame efforts is dependent on the type of survey conducted as described below:

2.C.1.a. Limestone Streams. For limestone stream surveys, two paired D-frame efforts are collected from each station - one from an area of fast current velocity and one from an area of slower current velocity within the same riffle.

2.C.1.b. Antidegradation Surveys. For Antidegradation surveys, it is necessary to characterize macroinvertebrate fauna communities from an area larger than a single riffle. Therefore, an Antidegradation survey station is defined as a stream reach of approximately 100 meters in length. At each station, six "D-frame efforts" are collected. Make an effort to spread the samples out over the entire reach. Choose the best riffle habitat areas and be certain to include areas of different depths (fast and slow) and substrate types that are typical of the riffle.

The resulting "D-frame efforts" (six for Anti-degradation, two for other survey types) are composited into one sample jar (or more as necessary). Care must be taken to minimize "wear and tear" on the collected organisms when compositing the materials. It is recommended that the benthic material be placed in a bucket and filled with water to facilitate gentle stirring and mixing. The sample is preserved in ethanol and returned to the lab for processing.

2.C.2. Sample Processing. Samples collected with a D-frame net are generally considered to be qualitative. However, the preserved samples can be processed in a manner which yields data that are "semi-quantitative" - data that were collected by qualitative methods but gives information that is almost statistically as strong as that collected by quantitative methods.

The following procedure is adapted from USEPA 1999 RBP methodology and used to process qualitative D-frame samples so that the resulting data can be analyzed using benthic macroinvertebrate biometric indices (or "metrics"). Equipment needed for the benthic sample processing are:

- 2 large laboratory pans gridded into 28 squares* (more gridded pans may be necessary depending on the size of the sample);
- an illuminated magnifying viewer;
- slips of paper (numbered from 1 to 28) for drawing random numbers;
- forceps (or any tools that can be used to pick floating benthic organisms); and
- grid cutters made from tubular material that approximates an inside area of 4 in^2 *.

* USEPA's (1989) gridding techniques suggested using "5 cm x 5 cm" (2" x 2") grids. Existing equipment consisted of 14" x 8" x 2" pans which were conducive to dividing into 2" x 2" grids and thus, contained 28 squares. The 4-in^2 grid cutters conform to these pan dimensions. While pan size is not critical, the number of grids (28) must be maintained if any basic density comparisons wish to be made between samples. Grid cutters (or similar sub-sampling devices) used with different sized pans should conform to the pans' grid dimensions.

The procedure described below begins with the premise that the collected samples have been properly composited according to the type of survey. For Antidegradation surveys, a station sample represents a composition of six D-frame efforts (collected from fast and slow riffle areas in a 100 meter reach). For Limestone surveys, a station sample is a composition of two D-frame efforts.

Following the steps listed below; process each composited D-frame sample to render a sub-sample size targeted for the specific survey type. The targeted sub-sample size for Antidegradation surveys is 200 benthic organisms and 300 for Limestone surveys (± 20% for each).

a. The composited sample is placed in a 28-square gridded pan (Pan1). It is recommended that the sample be rinsed in a standard USGS No. 35 sieve (or sieve bucket) to remove fine materials and residual preservative prior to sub-sampling.

b. The sample is gently stirred to disperse the contents evenly throughout Pan1 as thoroughly as possible. (In order to ease mixing and to minimize "wear-and-tear" on the more delicate organisms, water may be added to the pan to the depth of the sample material before stirring.)

c. Randomly select a grid using the 28 random number set and, using the grid cutters, remove the debris and organisms entirely from within the grid cutter (centered over the selected grid and "cut" into the debris) and place removed materials in a second gridded pan (Pan2).

    i. Float and pick, count, and sub-total all identifiable organisms (excluding pupae, larval bodies missing too many critical structures to render confident IDs, extremely small instar larvae, empty shells or cases, and non-benthic taxa) from each cut grid placed in Pan2. Repeat until at least 4 grids have been sub-sampled from Pan1. If, after 4 Pan1 grids have been sorted, the

sub-total is less than the targeted sub-sample (20 ± 20%), then continue to remove and sort grids one at a time until 200 organisms (± 20%) are obtained from Pan2. If the benthic organism yield from the 4 Pan1 grids exceeds the 200 ± 20% target (240+), then proceed to Step ii.

ii. With all of the 240+ identifiable organisms remaining in Pan2, randomly select one grid and "back count" (removing) all the organisms from that grid. Repeat one grid at a time until the bug count remaining in Pan2 satisfies the "200 ± 20%" rule.

d. If not identified immediately, the sub-sample should be preserved and properly labeled for future identification.

e. The benthic material remaining (Pan1) after the target sub-sample has been picked can be returned to its original sample jar and preserved. They shall be retained in accordance with QA retention times as specified for the respective survey type.

f. Any grid chosen must be picked in its entirety.

g. Record the final grid counts selected for each gridding phase (Pan1, Pan2, and Pan2 "back counting" as necessary) on the lab bench ID sheet for the sample.

<u>Processing larger, excessive amounts of D-frame sample debris</u>

Hopefully, the collector will rarely have very large amounts of D-frame materials to process. The reduction of large materials by careful removal, inspection, and rinsing in a bucket or using a sieve prior to field preservation or at the lab is encouraged. However, if the amount of material composited in the field jars exceeds the functional sorting capacity of Pan1, then follow this guidance:

- Evenly distribute the material between as many pans as necessary.
- From each pan (Pan1a, Pan1b, etc.), remove debris and organisms from 4 random grids and place in Pan2 as described in Step 2.C.2.c above.
- Once the required 4 grids from each Pan1 have been placed in Pan2, evenly and gently redistribute the materials as in Step 2.C.2.b.
- Then, resume processing, again as described in Step 2.C.2.c, selecting a grid from Pan2 and placing the materials into a gridded Pan3.
- Process this material and repeat as described in Step 2.C.2.c.i until the targeted 200 ± 20% sub-sample is obtained from Pan3.
- If, after processing 4 grids, the +20% upper limit (240+) is obtained, follow "back counting" method in Step 2.C.2.c.ii.
- Once the targeted sub-sample is reached, continue with Step 2.C.2.d.

2.D. Identification

2.D.1. Taxonomic Level. The level of identification for most aquatic macroinvertebrates will be to genus. Presently, the identification of Chironomidae, or midges, is to the family level. Some individuals collected will be immature and not exhibit the characteristics necessary for confident identification. Therefore, the lowest level of taxonomy attainable will be sufficient. Certain groups, however, may be identified to a higher taxonomic level as follows:

Snails (Gastropoda) - Family
Clams, mussels (Bivalvia) - Family
Flatworms (Turbellaria)
    identifiable planariids - genus
    or Family Planariidae
    others – Class Turbellaria
Segmented worms (Annelida)
    aquatic earthworms & tubificids - Class Oligochaeta
    leeches - Class Hirudinea
    Moss animacules - Phylum Bryozoa
Proboscis worms – Phylum Nemertea
Roundworms - Phylum Nematoda
Water mites- “Hydracarina” (an artificial taxonomic grouping of several mite superfamilies)

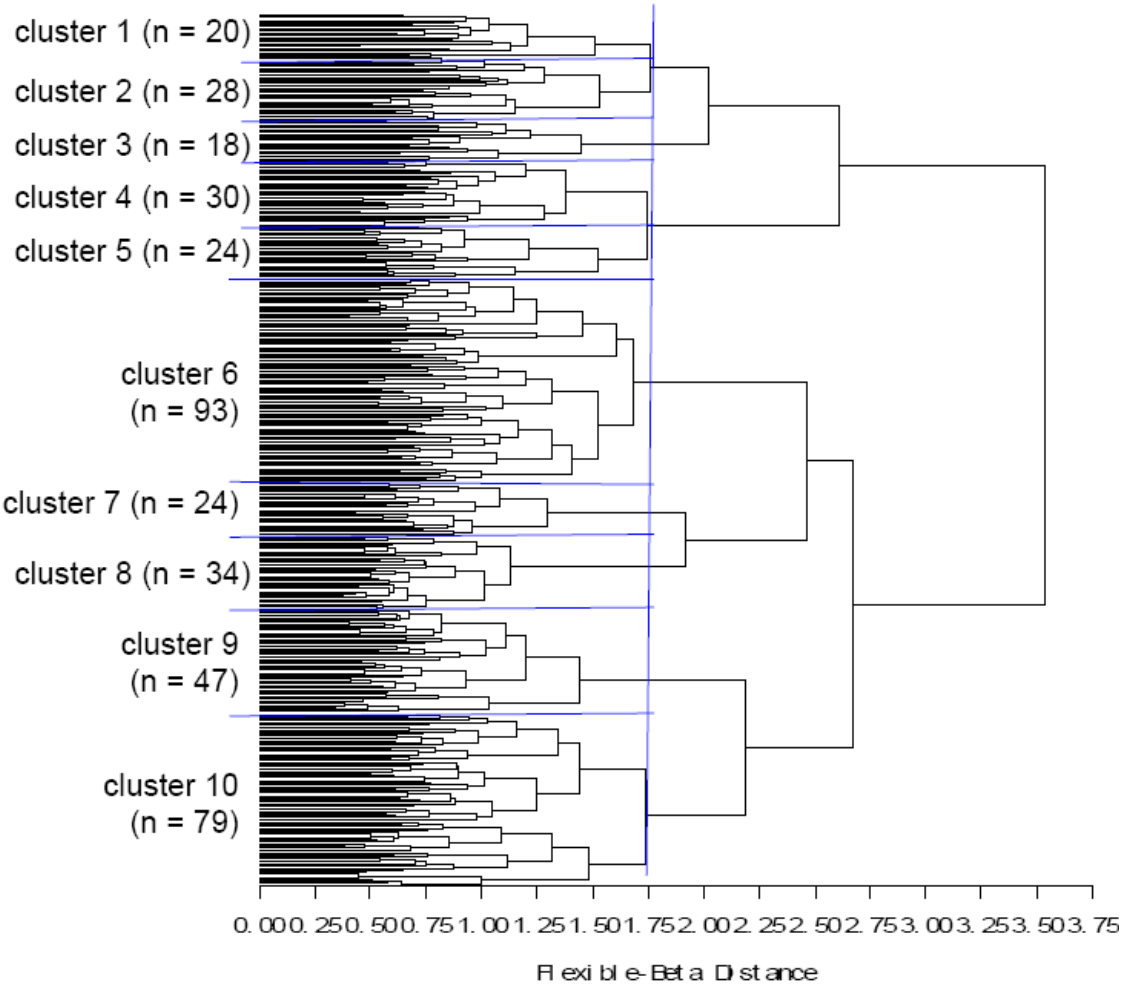
2.D.2. Verifications. For Quality Assurance purposes, certain laboratory invertebrate processing procedures should be checked routinely. Normally, a colleague may perform these spot checks. These include the floating/picking steps, taxonomic identifications, and total taxa list scans:

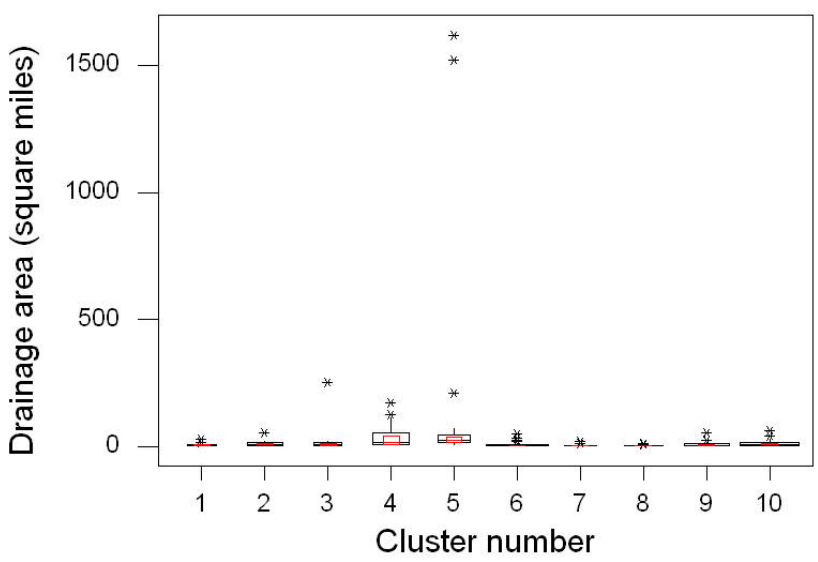
a. Sorting. After the floating and picking has been completed for samples that require this treatment (Pa-RBP, Surber-type, multi-plate, and grab samples), the residue should be briefly scanned before discarding to assure that the sample has been sufficiently “picked”. This should be done for 10% of the samples (or at least one sample) per survey.

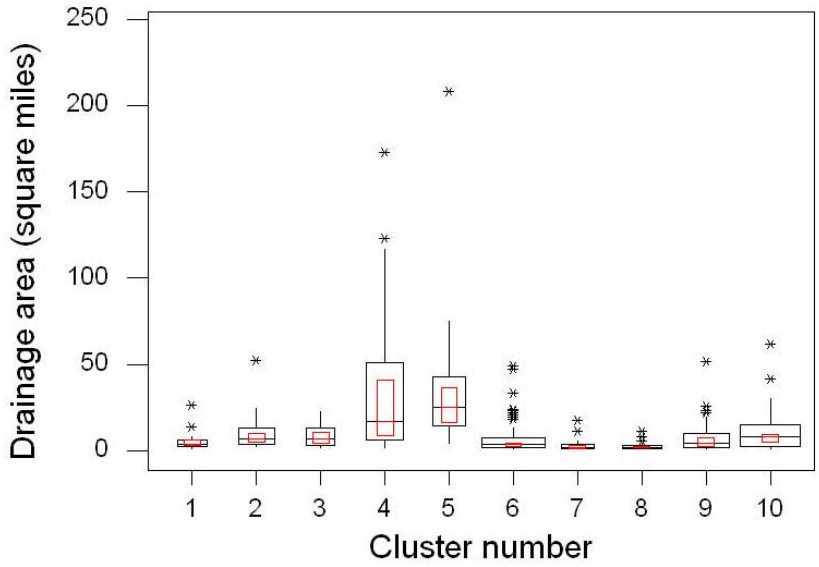
b. Identification. For samples not involving litigation or enforcement issues, laboratory bench ID sheets for all samples should be reviewed. Any unusual taxa or taxa that are not typical to the type of stream or water quality condition that was surveyed, should be checked. For samples involving legal issues, representative specimens of each taxon may need to be verified by independent expert taxonomists.

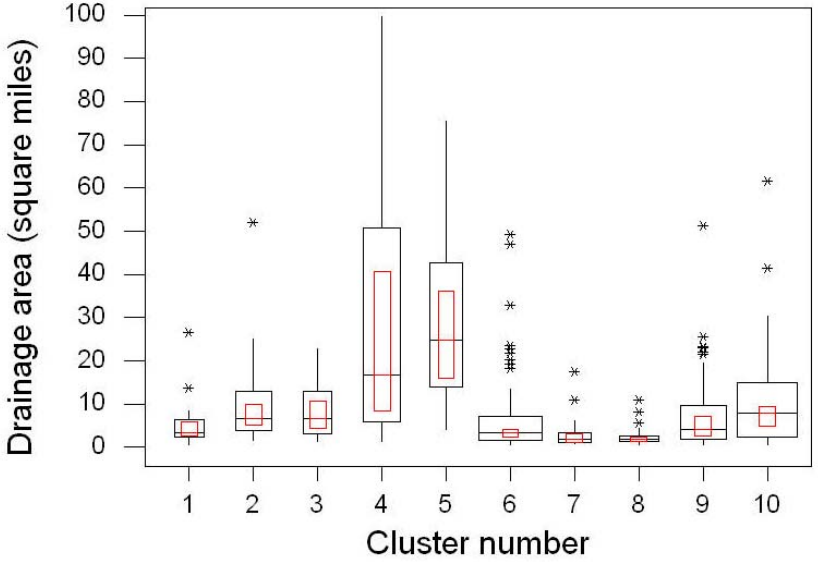
Appendix B – Graphical Cluster Analysis

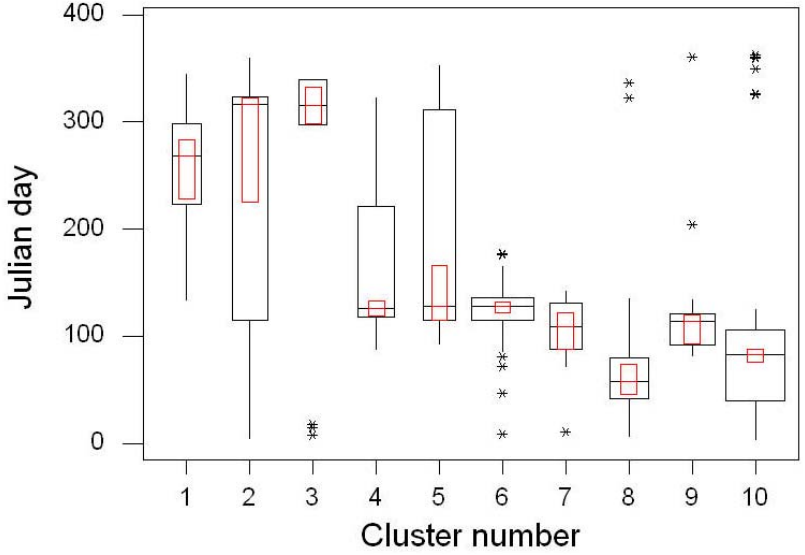
Graphical analyses of the cluster groups is presented below by: drainage area; Julian day; elevation; latitude; longitude; river basin; habitat index; and land use index. The clusters showed marked differences in certain parameters, most notably drainage area and sampling season. As stated in the text of the report, the cluster tree was analyzed at the level of ten clusters. The cluster tree is reproduced immediately below for reference.

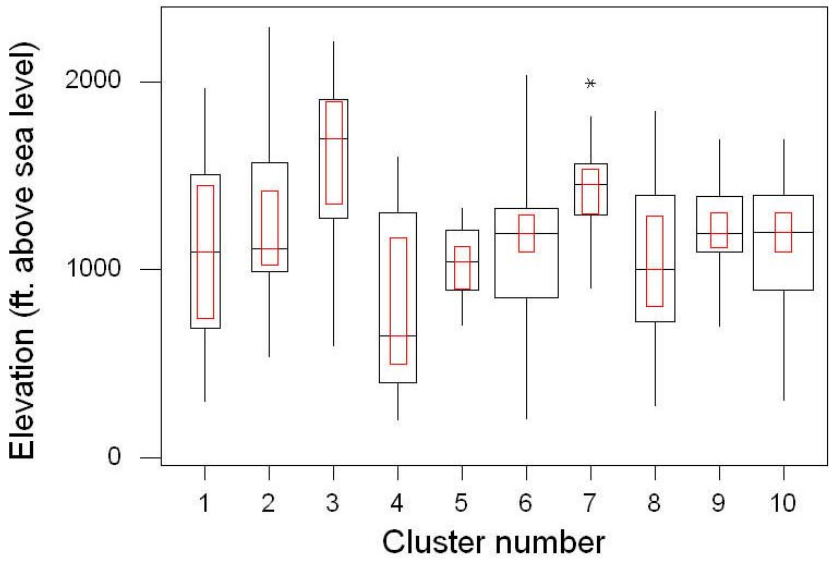


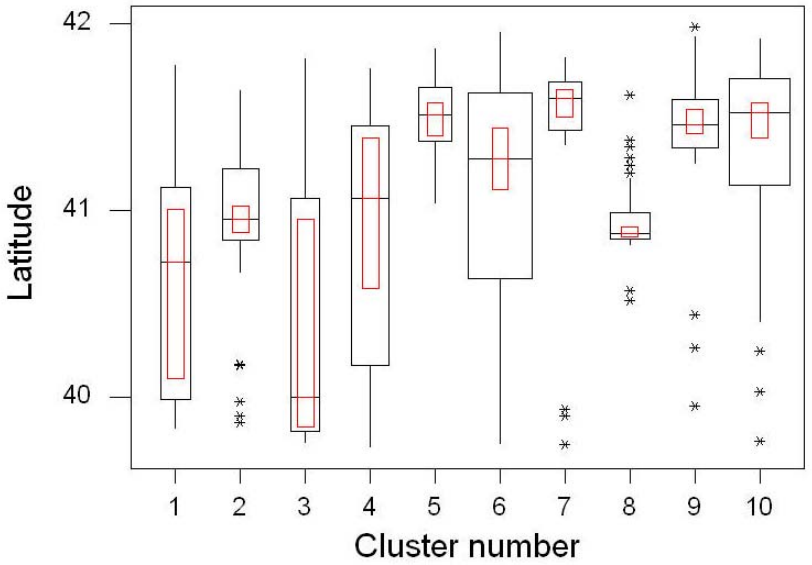

Drainage area (square miles)
0
500
1000
1500
1 2 3 4 5 6 7 8 9 10
Cluster number

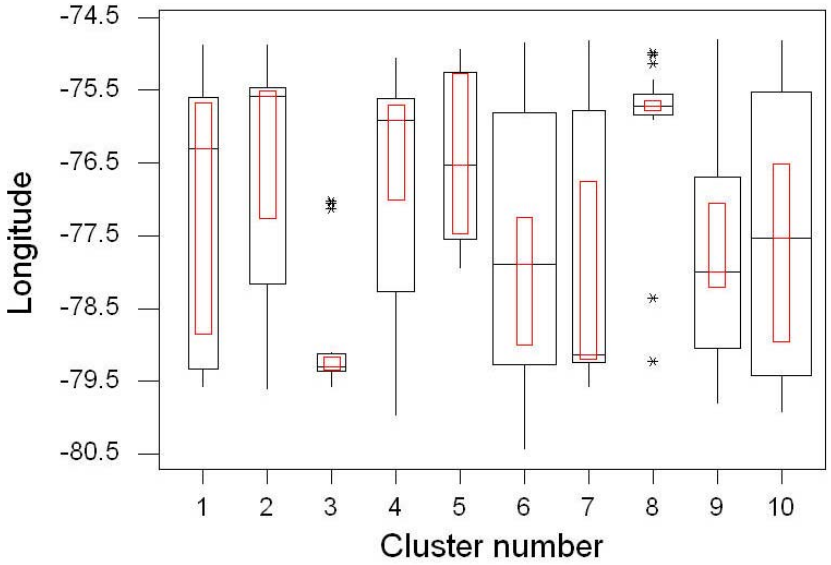

Drainage area (square miles)
0
50
100
150
200
250
1 2 3 4 5 6 7 8 9 10
Cluster number

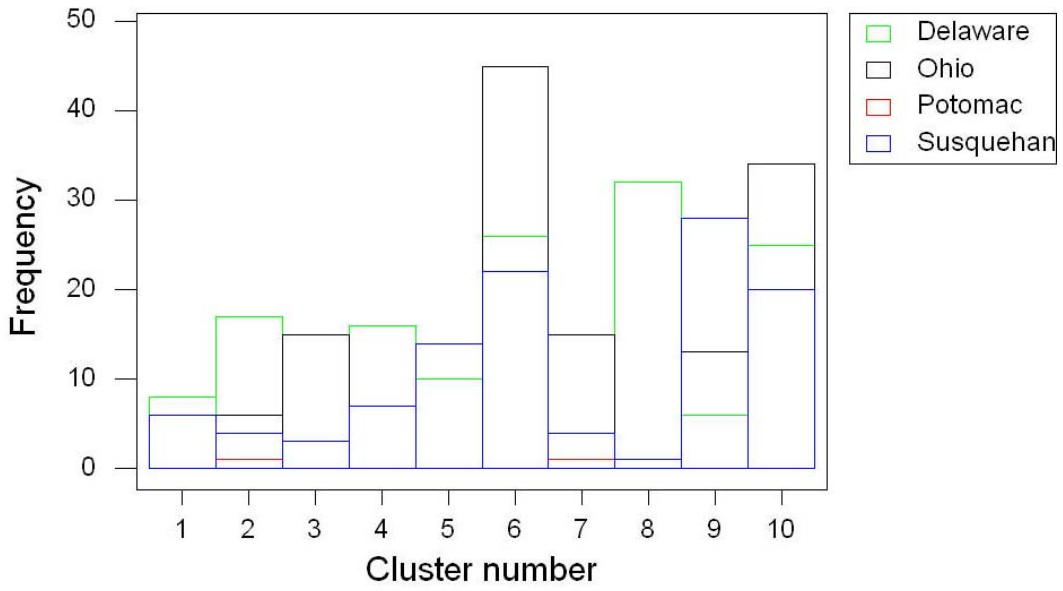

Drainage area (square miles)
0
10
20
30
40
50
60
70
80
90
100
1 2 3 4 5 6 7 8 9 10
Cluster number

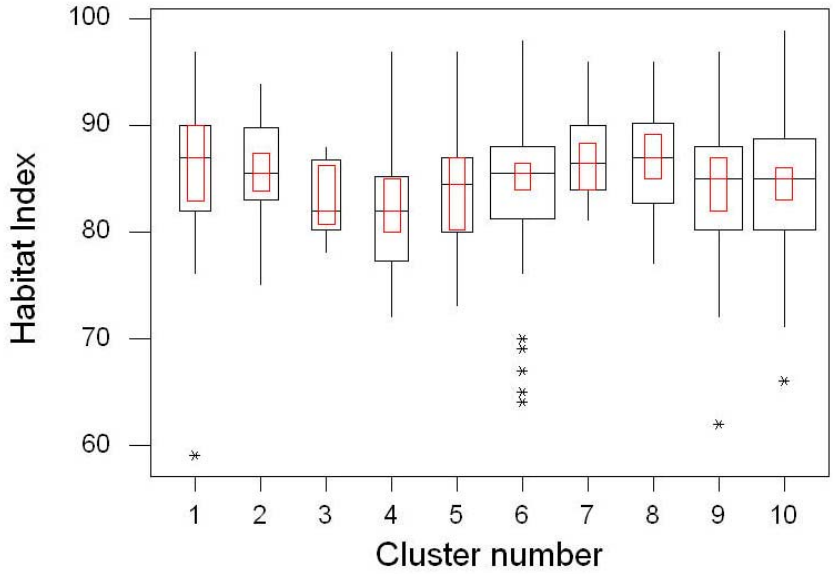

Julian day
0
100
200
300
400
1 2 3 4 5 6 7 8 9 10
Cluster number

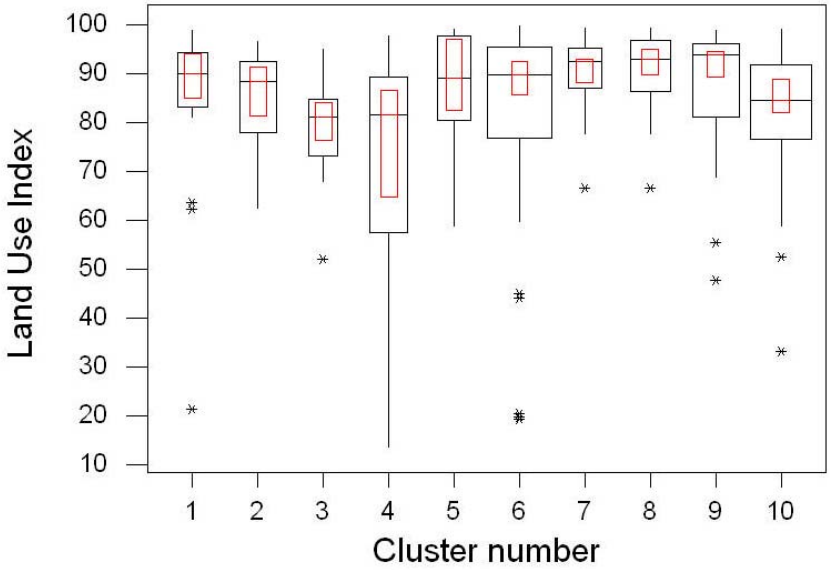

Elevation (ft. above sea level)
2000
1000
0
1 2 3 4 5 6 7 8 9 10
Cluster number


Latitude
42
41
40
1 2 3 4 5 6 7 8 9 10
Cluster number


Longitude
-74.5
-75.5
-76.5
-77.5
-78.5
-79.5
-80.5
1 2 3 4 5 6 7 8 9 10
Cluster number

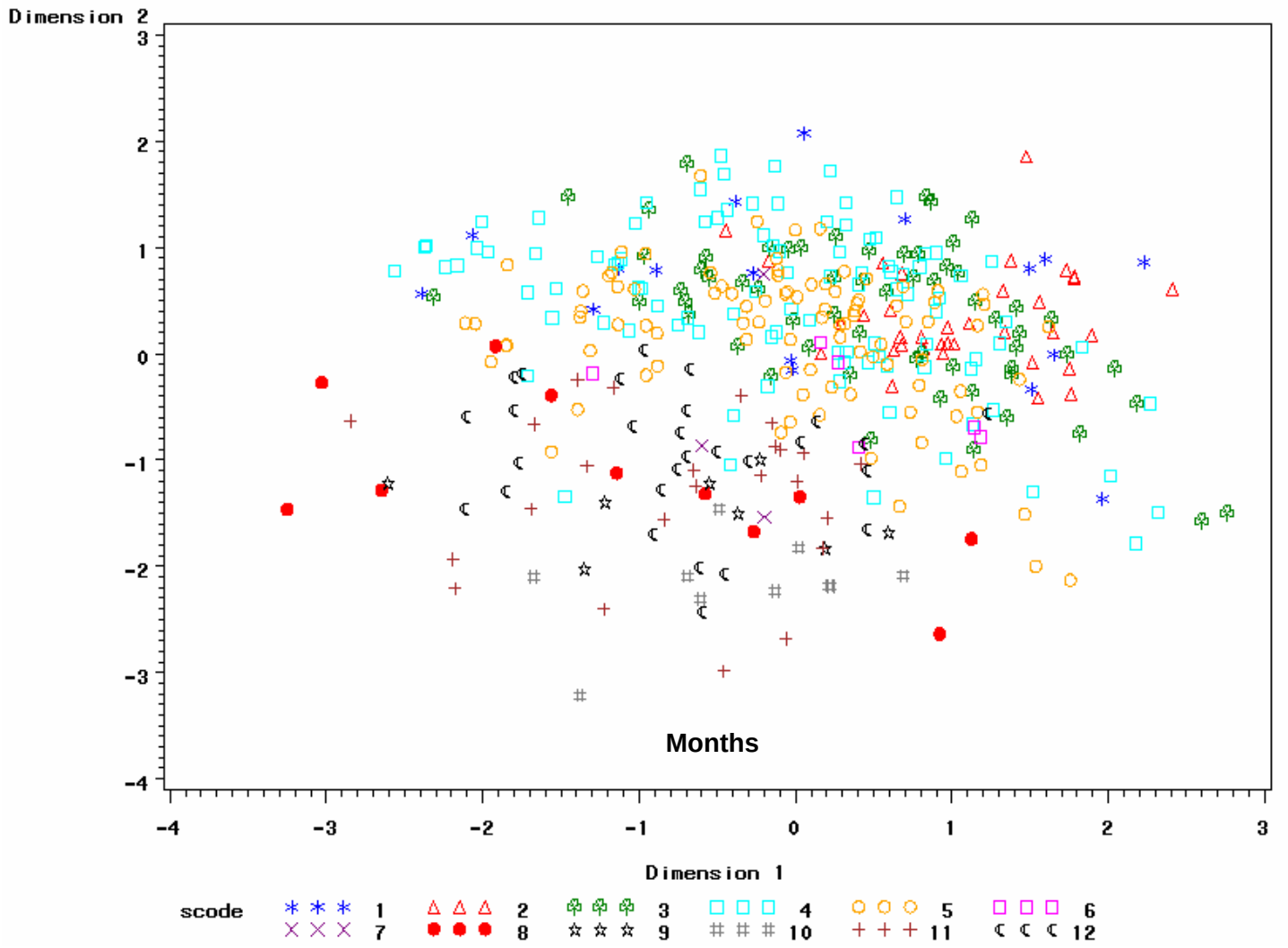

Frequency
Cluster number
Delaware
Ohio
Potomac
Susquehan

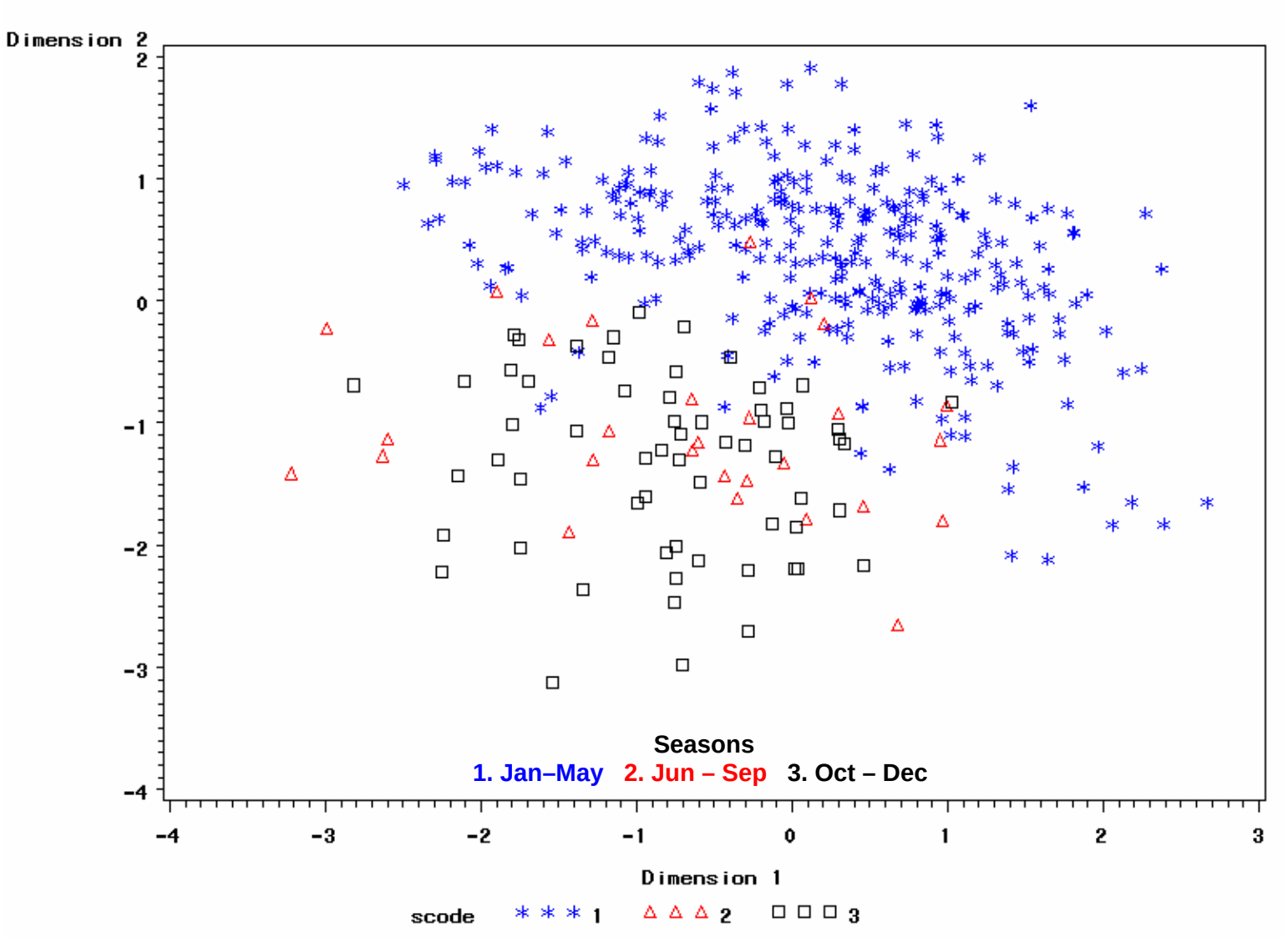

Habitat Index
Cluster number

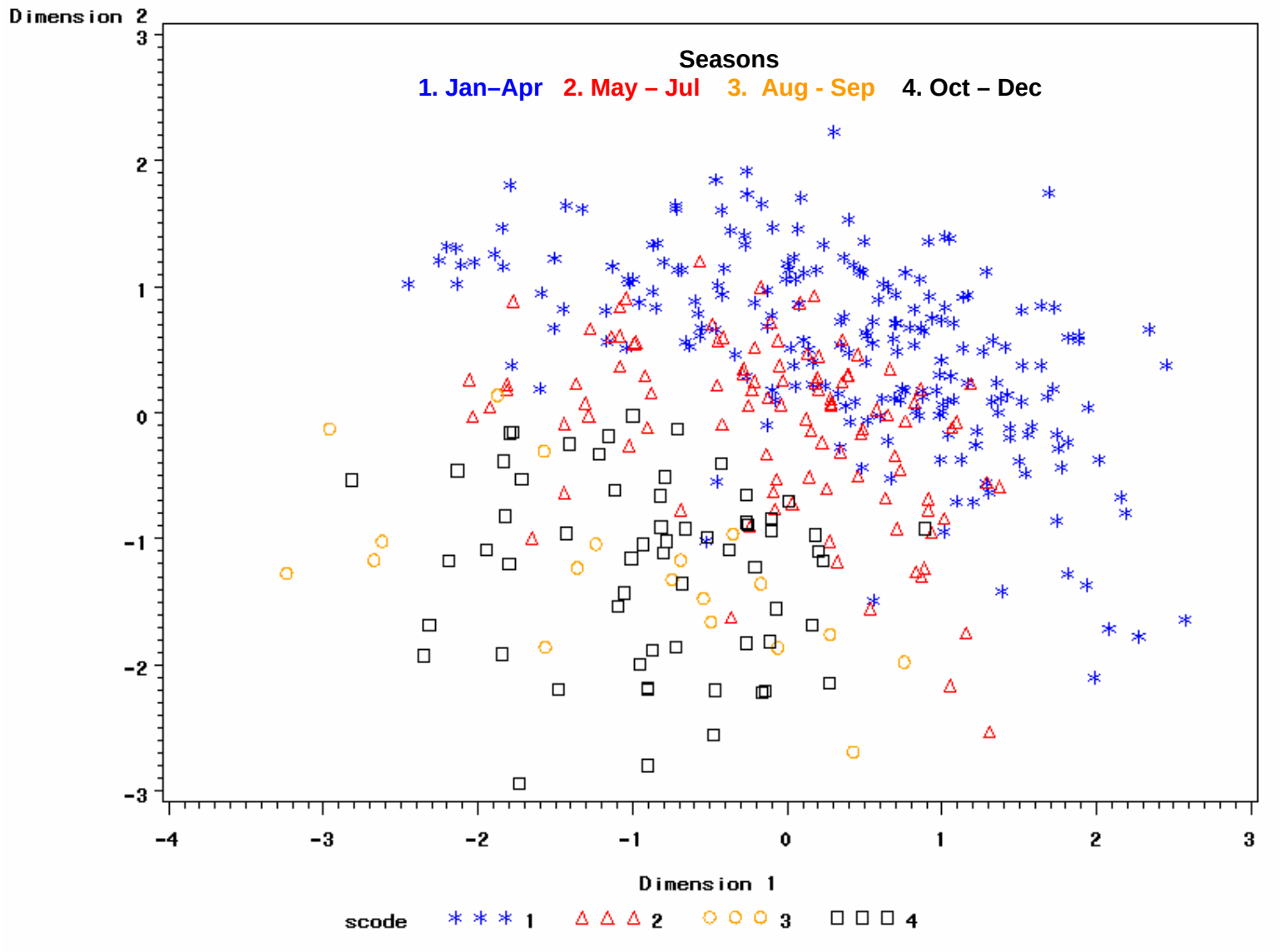

Land Use Index
Cluster number

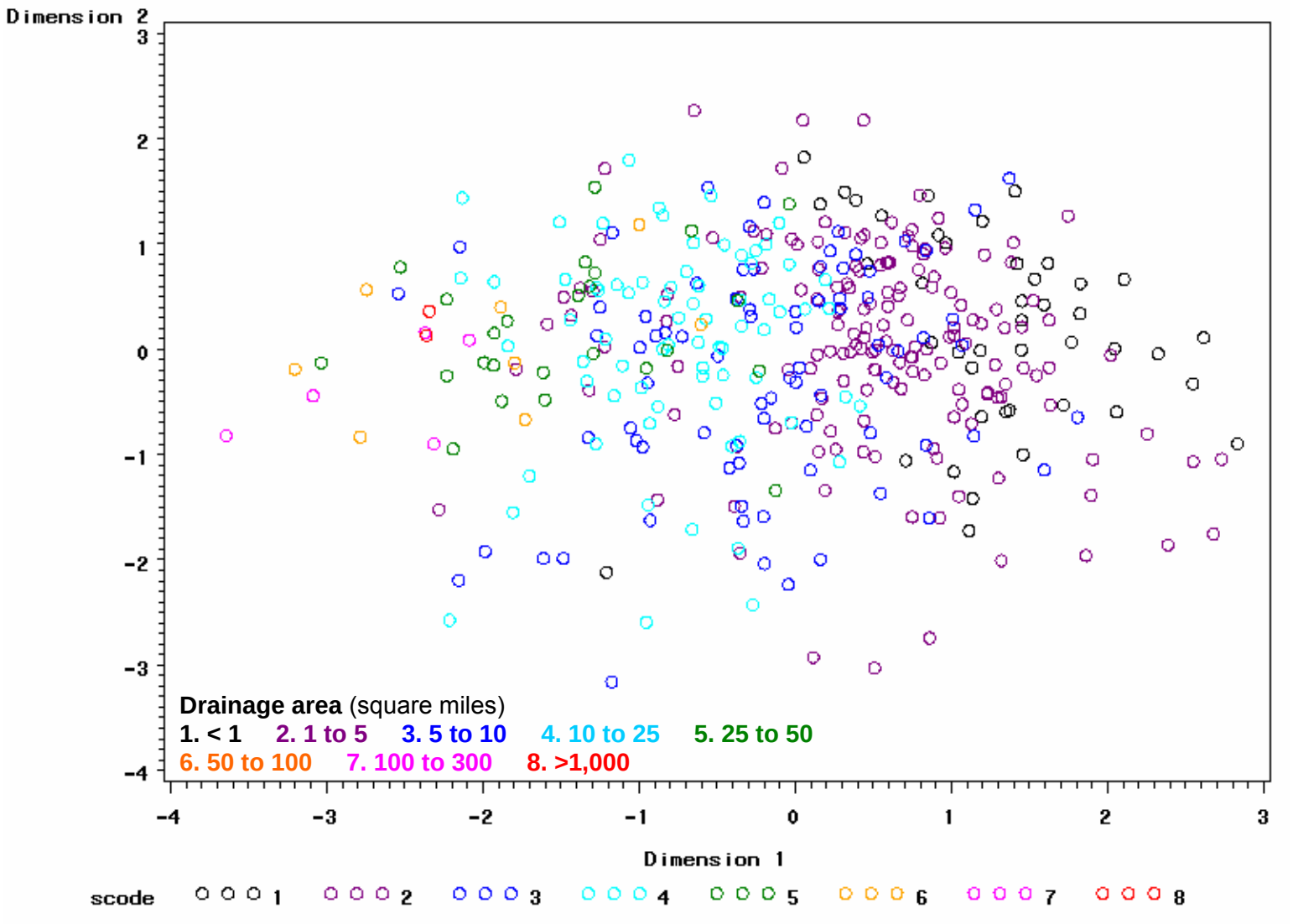
Appendix C – NMDS Ordination Plots

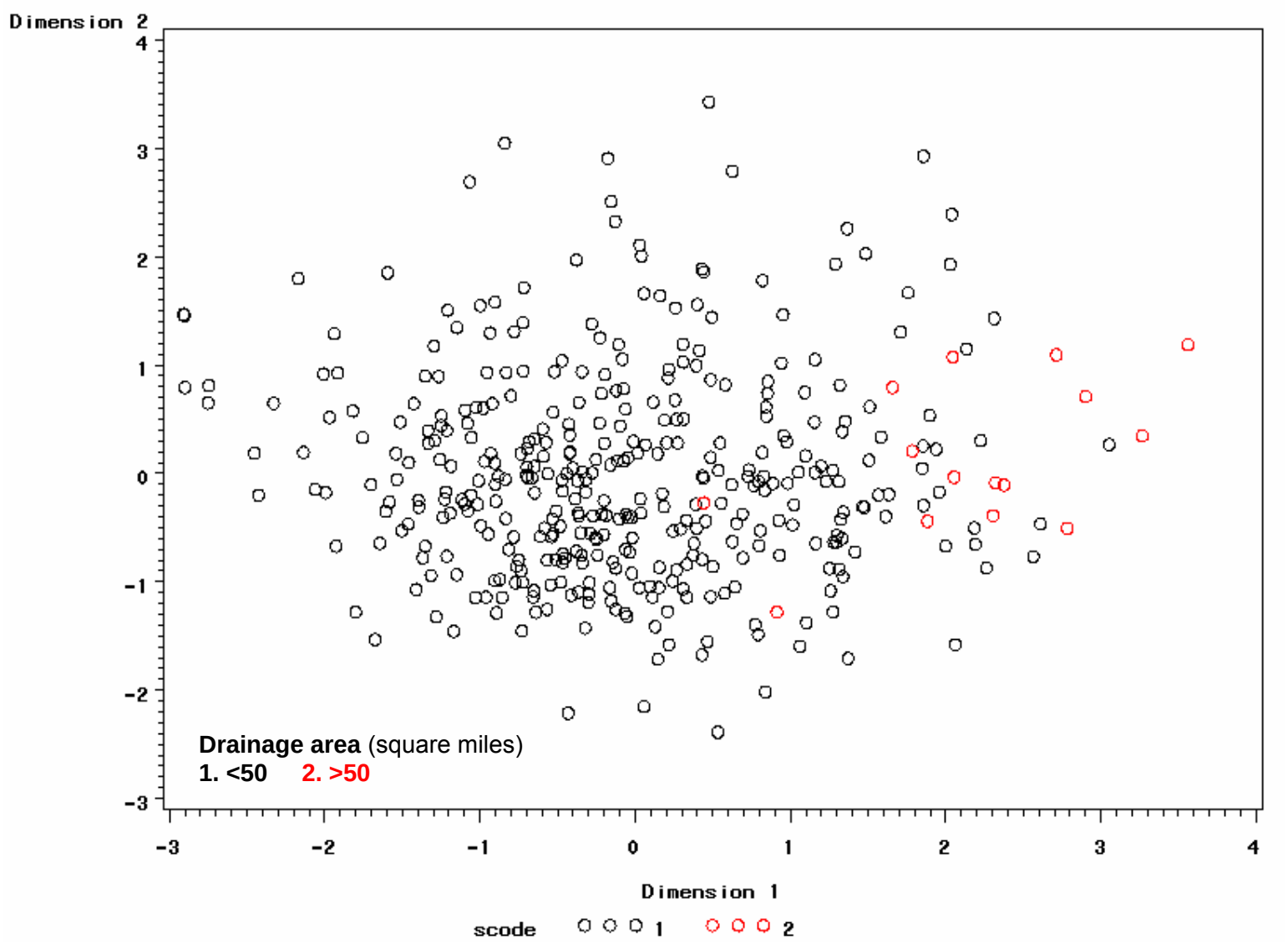
The plots in this appendix show the first two NMDS dimensions with samples symbolized according to various classification schemes. These plots were generated using natural log-transformed abundance data of the 134 most common taxa found in tier A samples (see main text for NMDS discussion and procedural details).

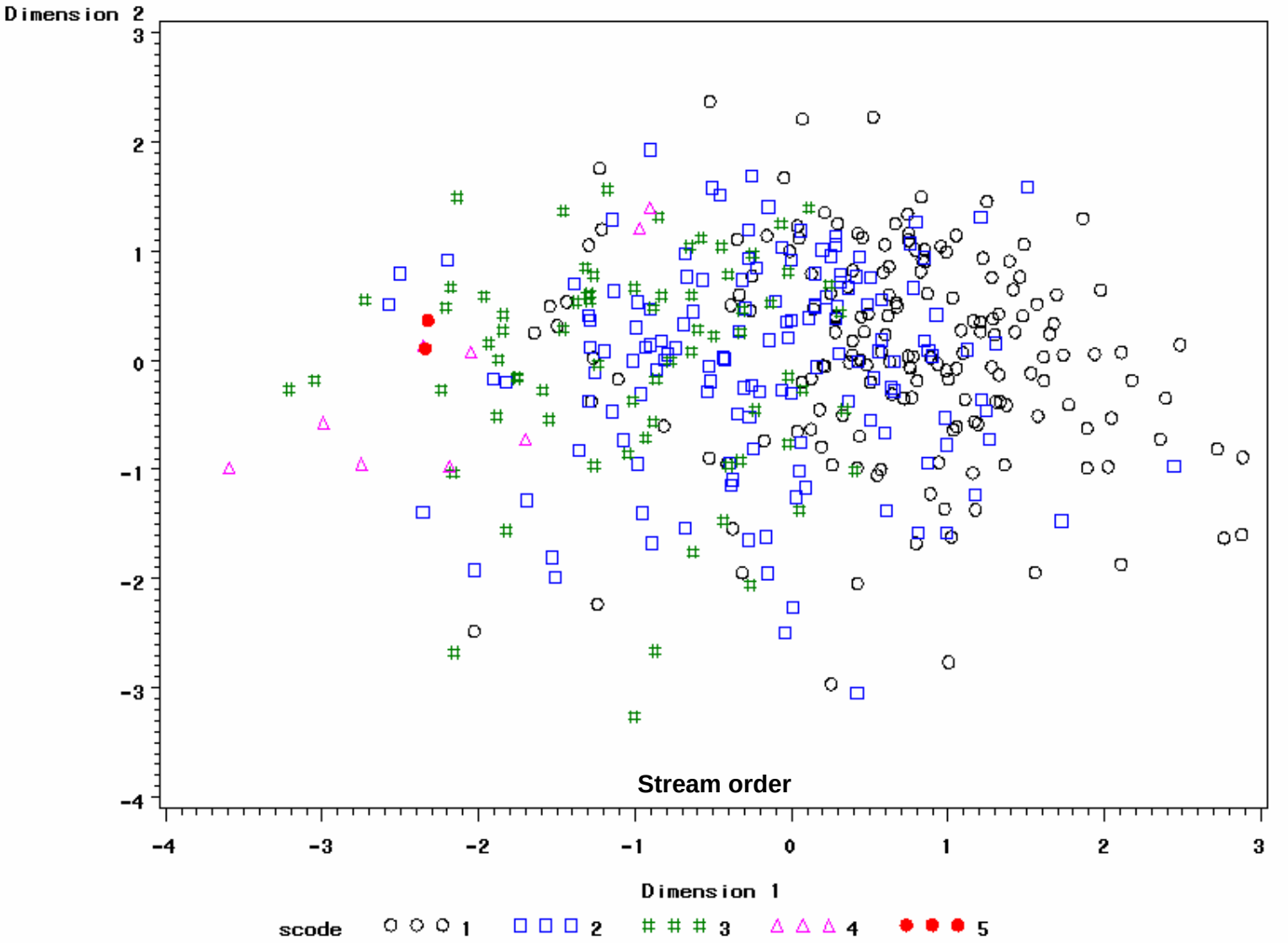


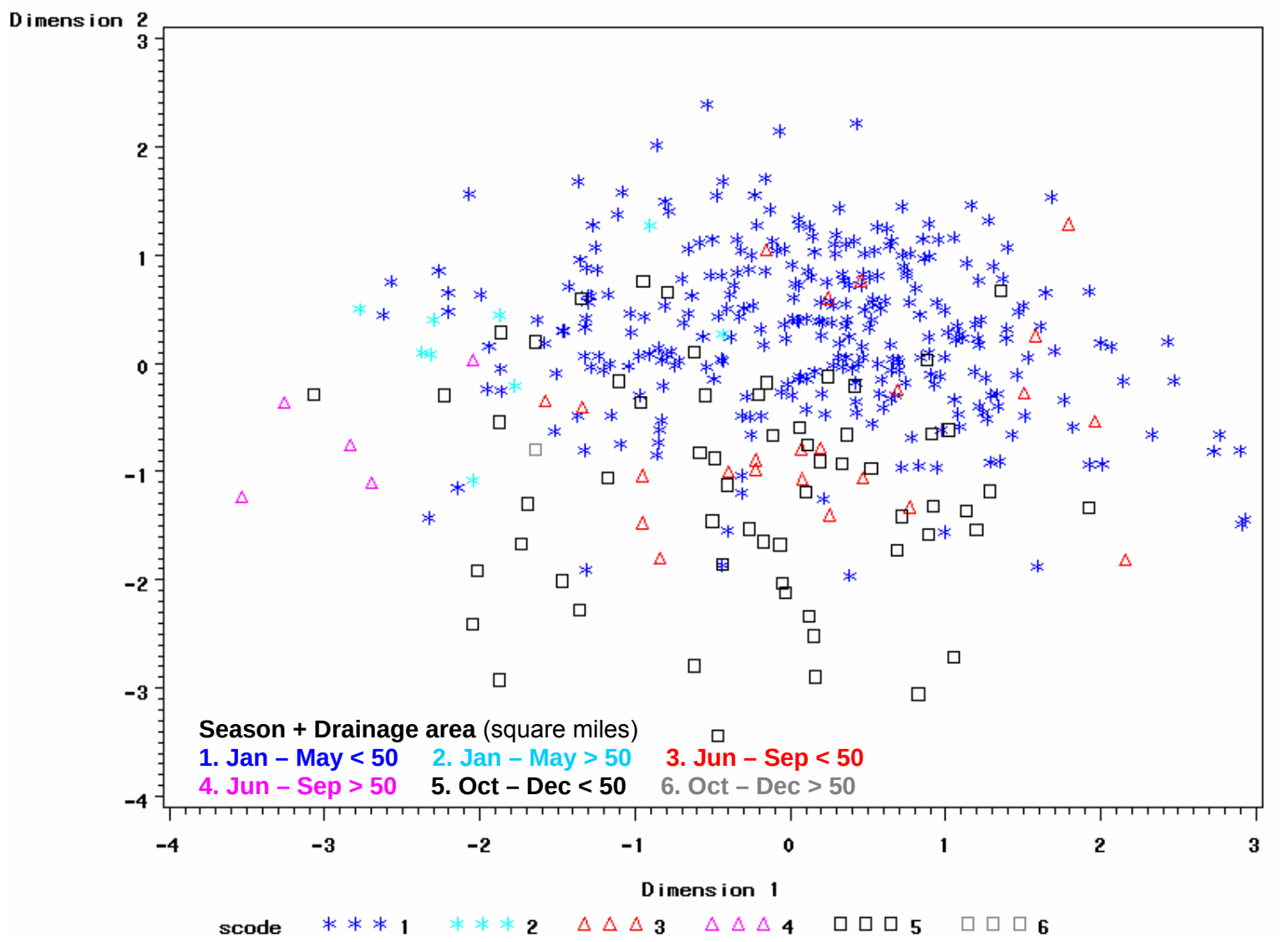

Dimension 2
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
Dimension 1
Seasons
1. Jan–May
2. Jun – Sep
3. Oct – Dec
scode
1
2
3

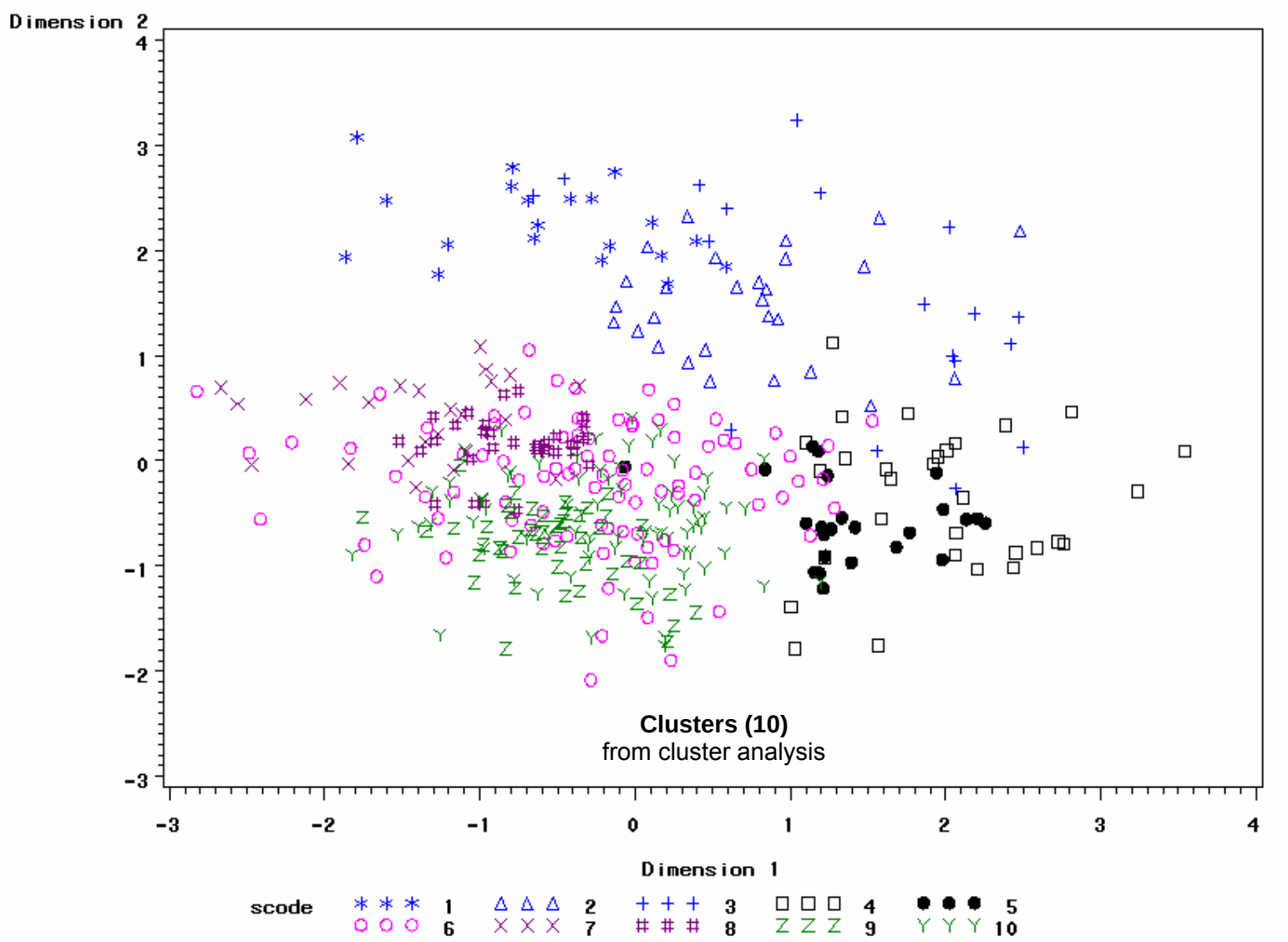

Seasons
1. Jan–Apr 2. May – Jul 3. Aug - Sep 4. Oct – Dec
Dimension 2
Dimension 1
scode 1 2 3 4

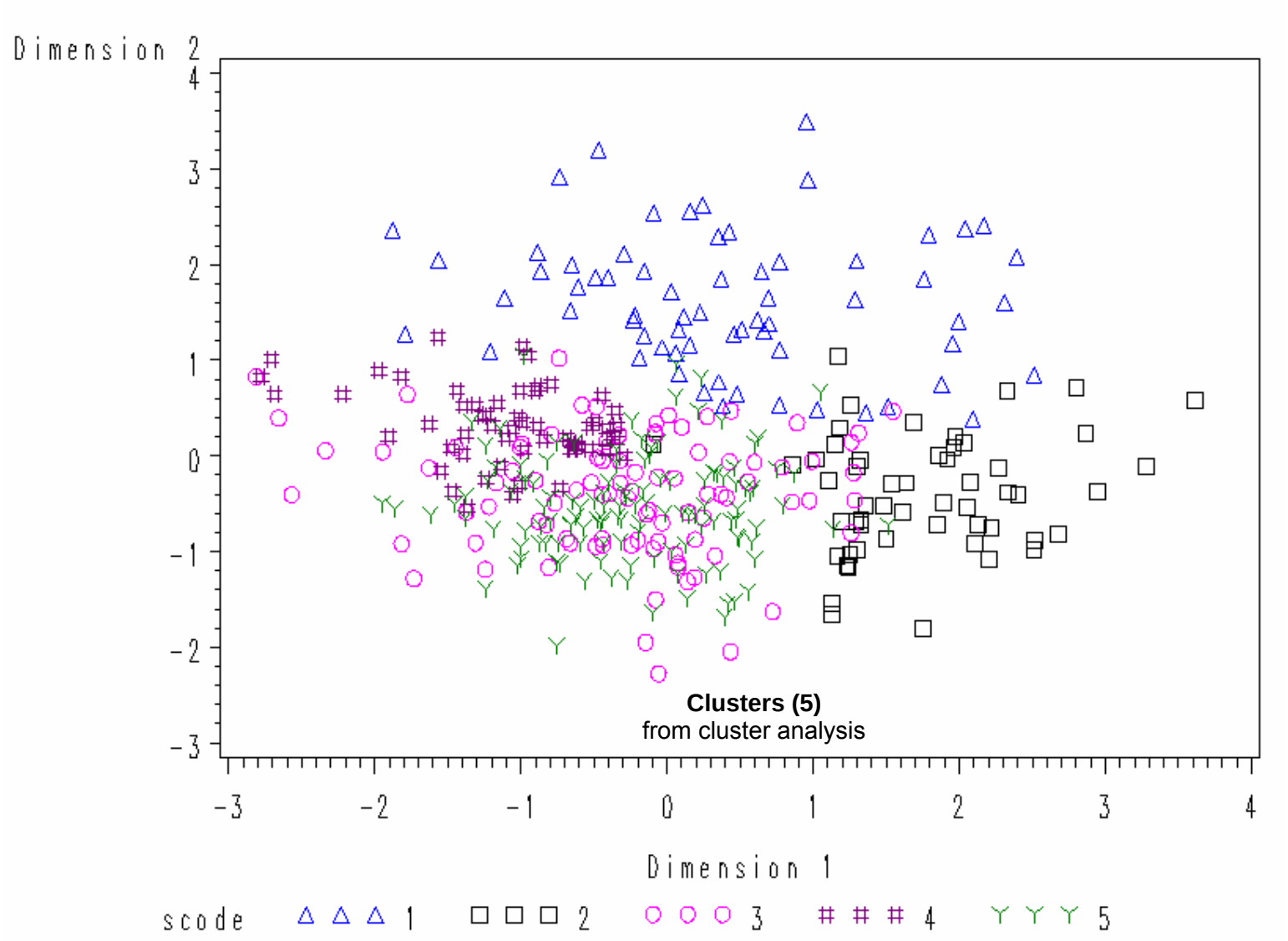

Dimension 2
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
Dimension 1
Drainage area (square miles)
1. < 1
2. 1 to 5
3. 5 to 10
4. 10 to 25
5. 25 to 50
6. 50 to 100
7. 100 to 300
8. >1,000
scode
1
2
3
4
5
6
7
8


Dimension 2
4
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4
Dimension 1
Drainage area (square miles)
1. <50
2. >50
scode
1
2

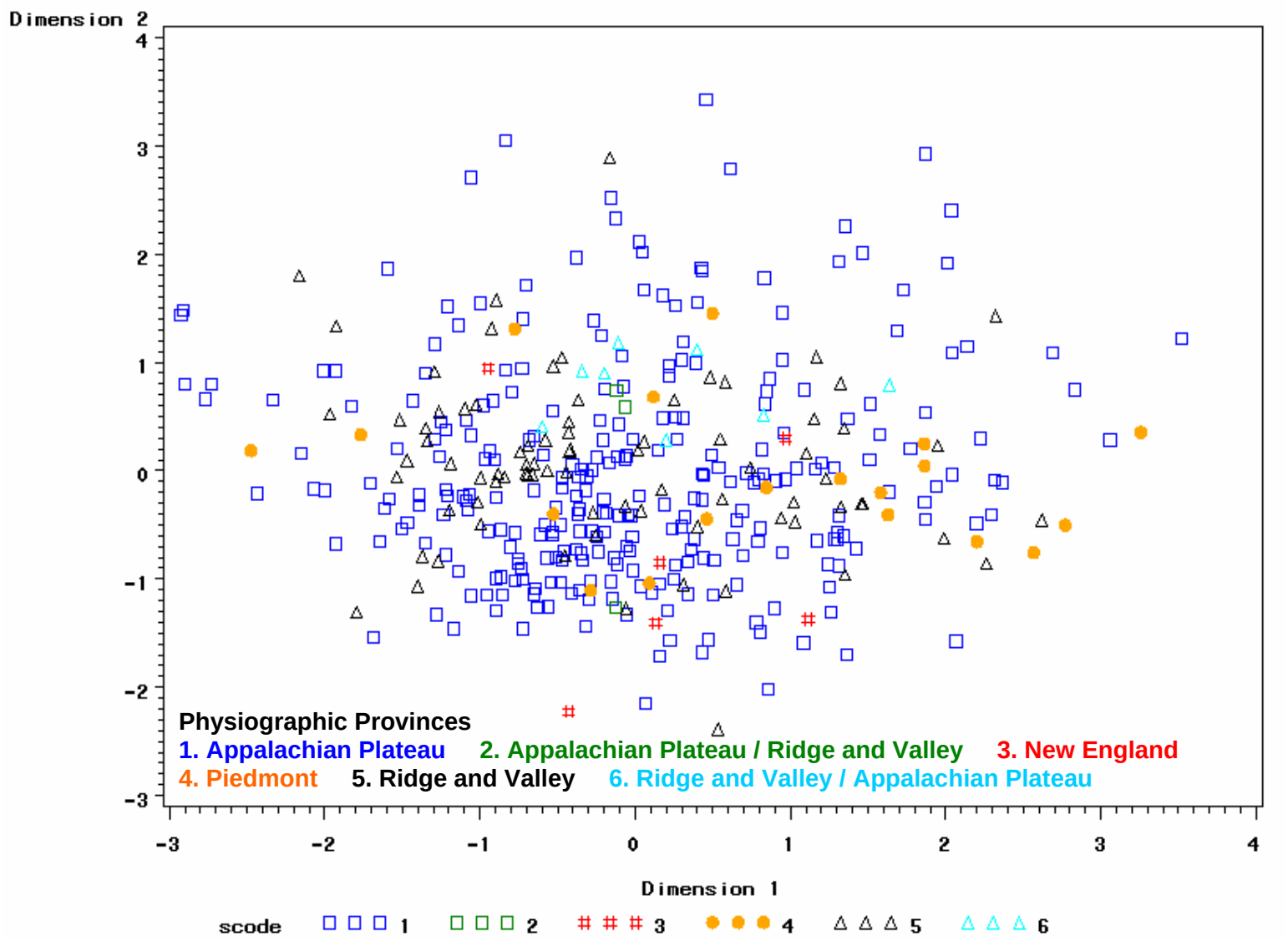

Dimension 2
3
2
1
0
-1
-2
-3
-4
Stream order
-4
-3
-2
-1
0
1
2
3
Dimension 1
scode
1
2
3
4
5

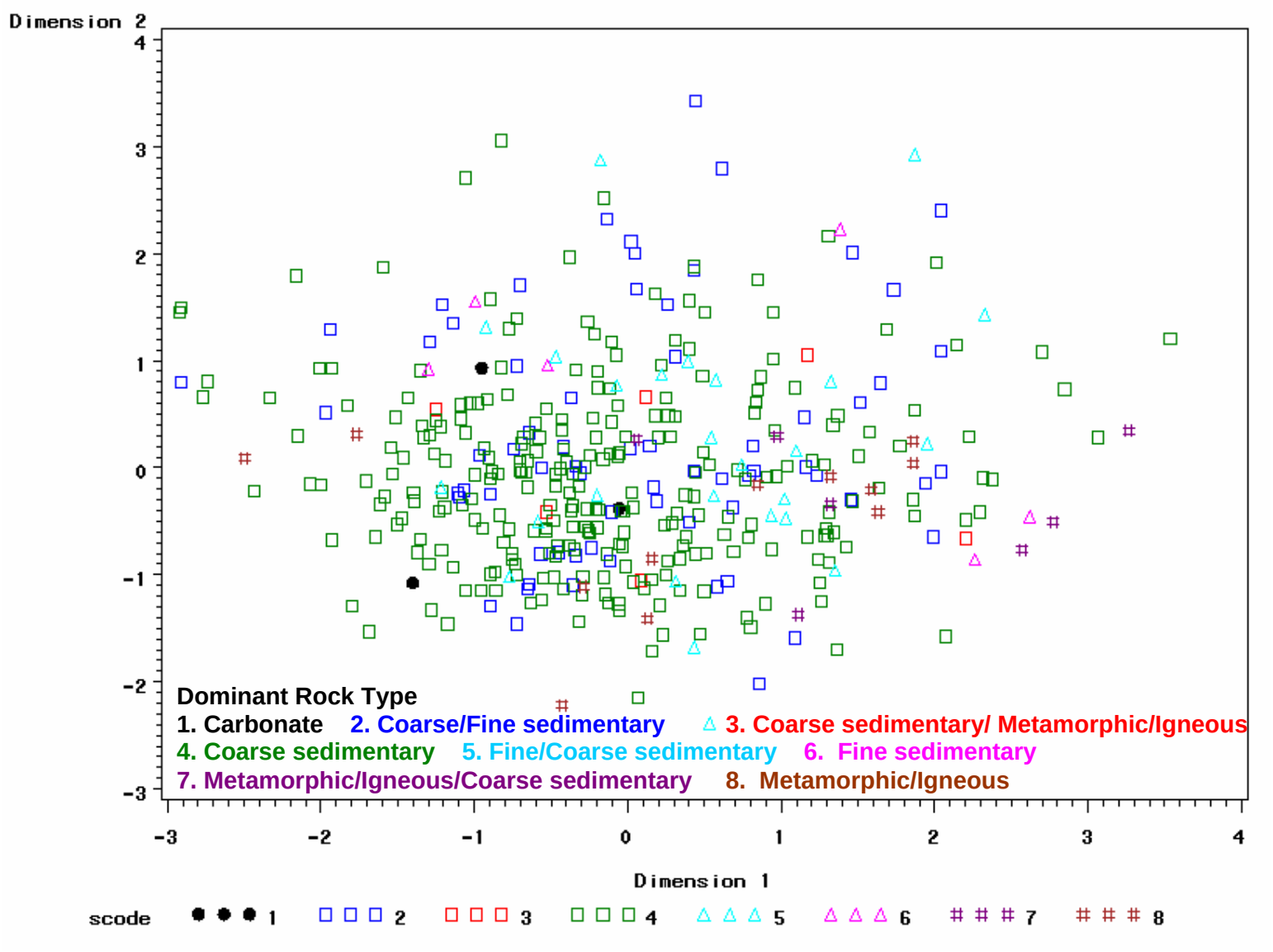

Dimension 2
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
Dimension 1
Season + Drainage area (square miles)
1. Jan – May < 50
2. Jan – May > 50
3. Jun – Sep < 50
4. Jun – Sep > 50
5. Oct – Dec < 50
6. Oct – Dec > 50
scode
1
2
3
4
5
6

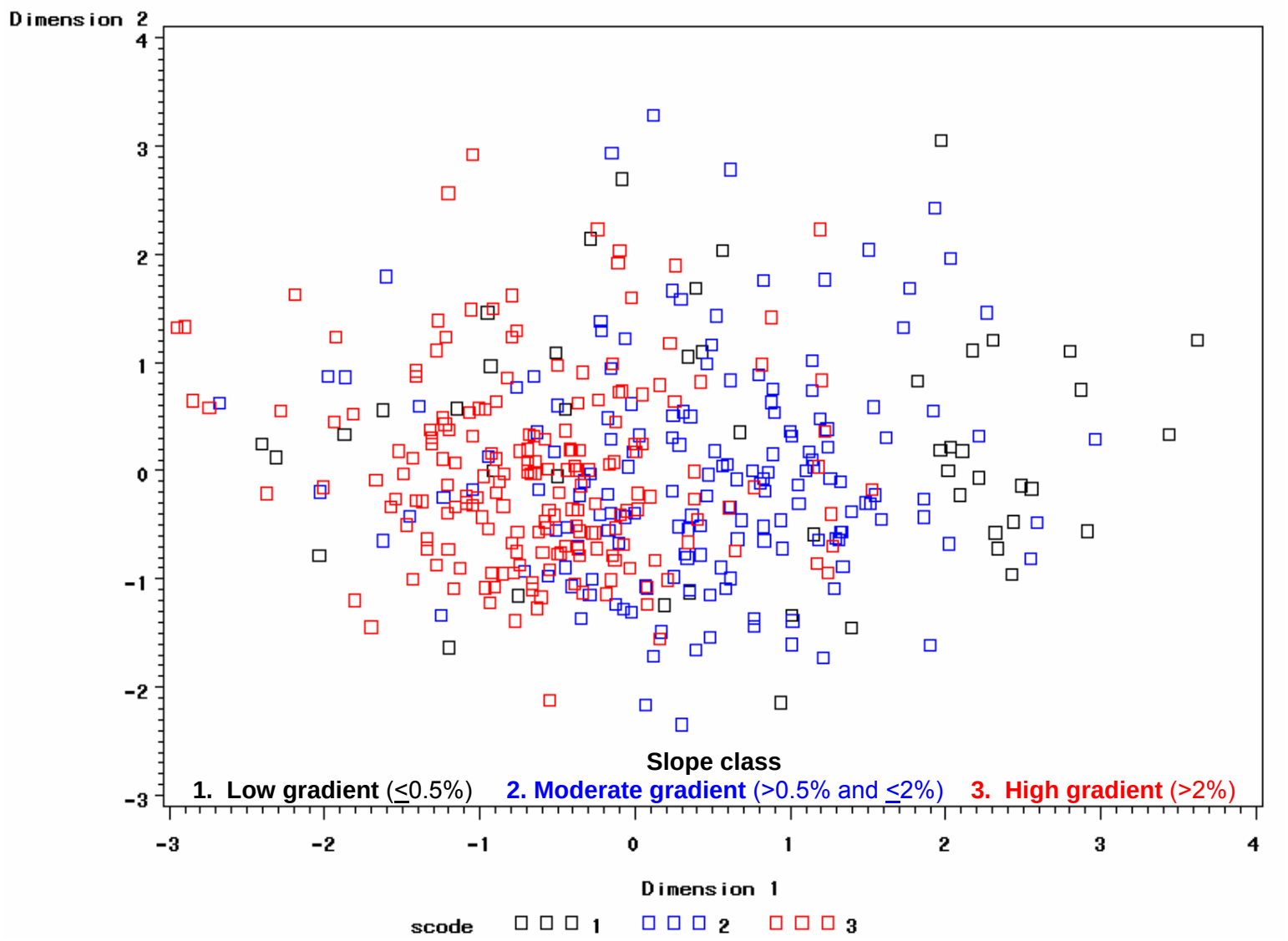

Dimension 2
4
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4
Dimension 1
Clusters (10)
from cluster analysis
scode
1
2
3
4
5
6
7
8
9
10

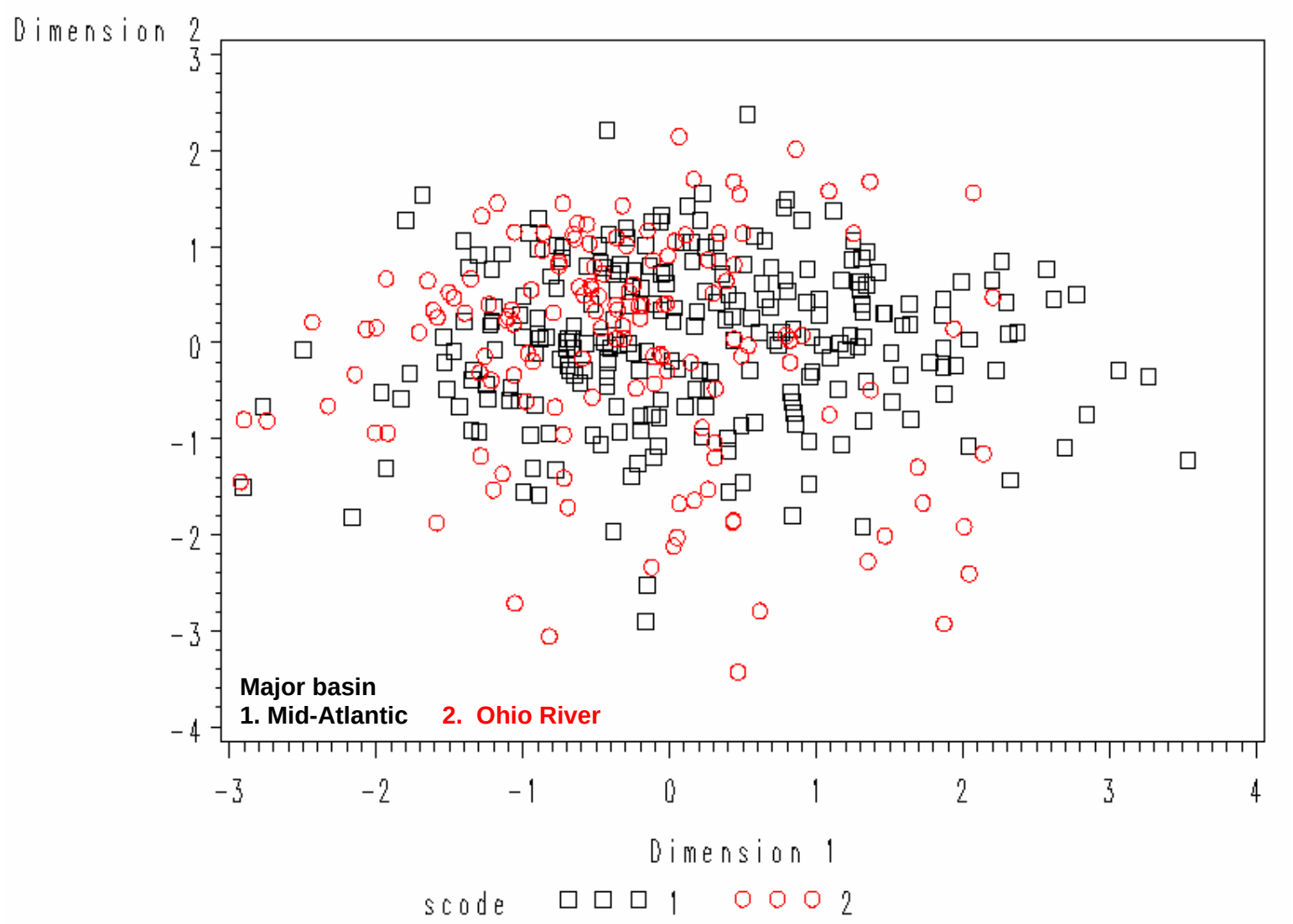


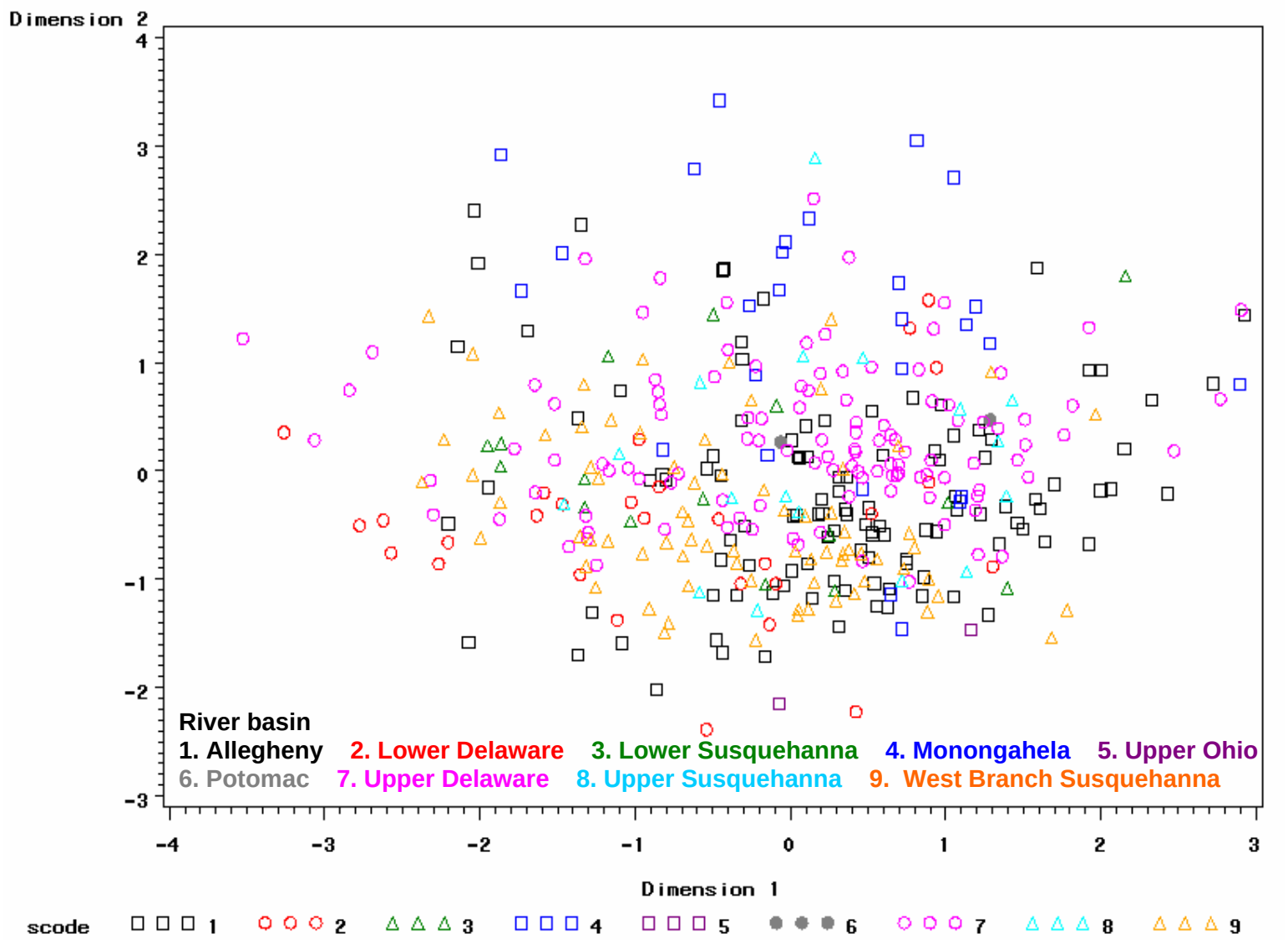
Clusters (5)
from cluster analysis

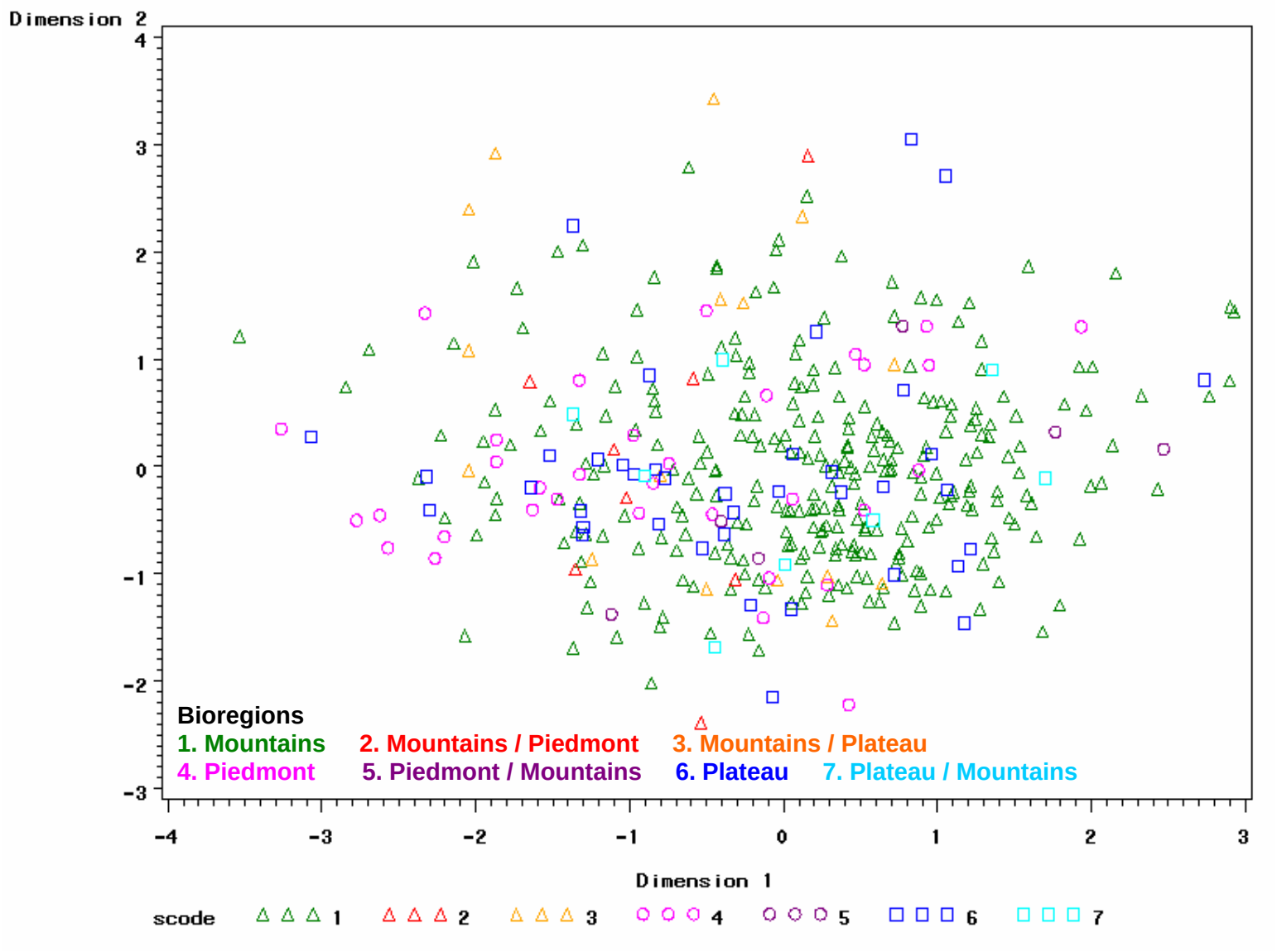

Dimension 2
4
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4
Dimension 1
Physiographic Provinces
1. Appalachian Plateau
2. Appalachian Plateau / Ridge and Valley
3. New England
4. Piedmont
5. Ridge and Valley
6. Ridge and Valley / Appalachian Plateau
scode
1
2
3
4
5
6

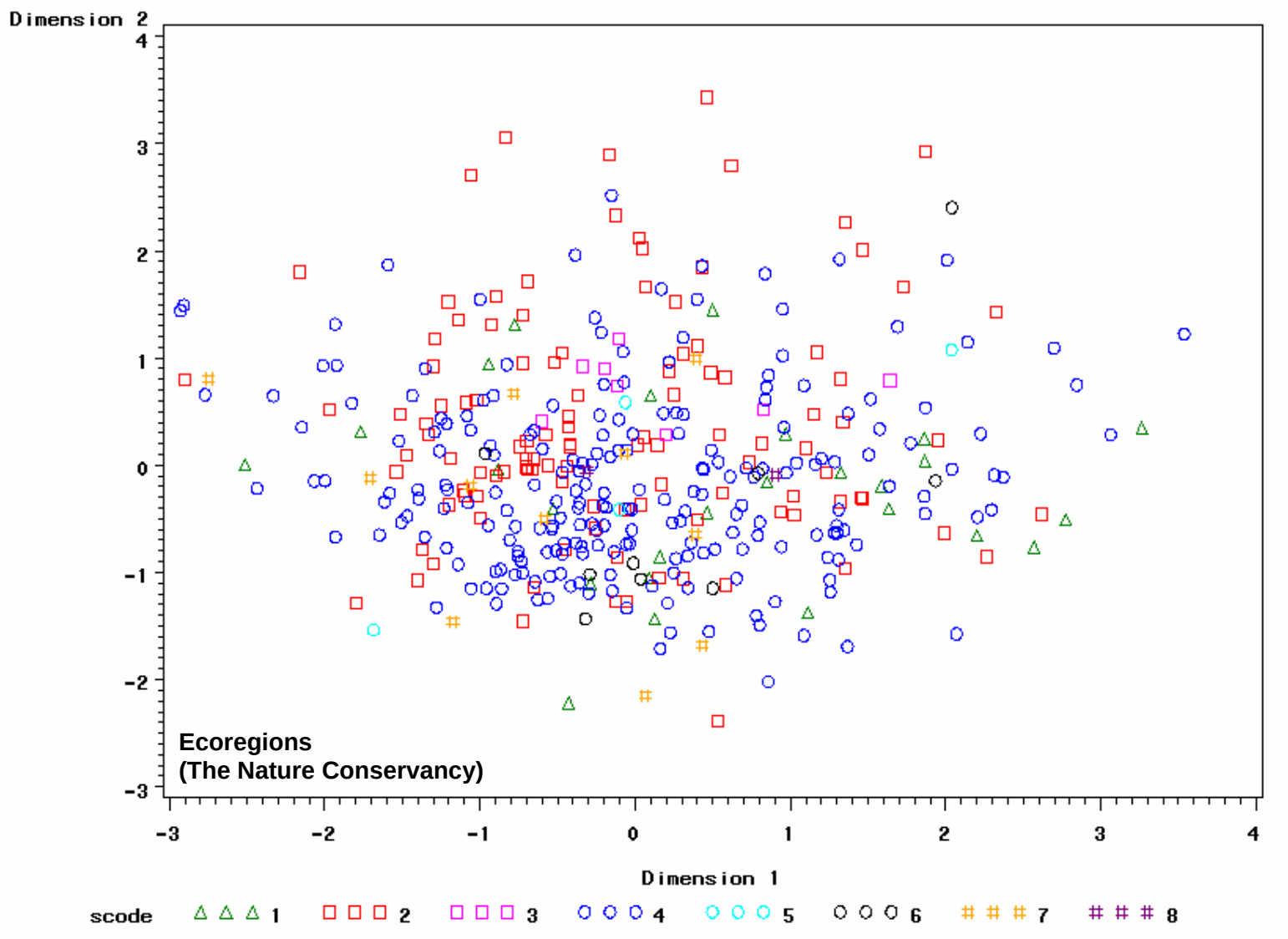

Dimension 2
4
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4
Dimension 1
Dominant Rock Type
1. Carbonate
2. Coarse/Fine sedimentary
3. Coarse sedimentary/ Metamorphic/Igneous
4. Coarse sedimentary
5. Fine/Coarse sedimentary
6. Fine sedimentary
7. Metamorphic/Igneous/Coarse sedimentary
8. Metamorphic/Igneous
scode
1
2
3
4
5
6
7
8


Dimension 2
4
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4
Dimension 1
Slope class
1. Low gradient (≤0.5%)
2. Moderate gradient (>0.5% and ≤2%)
3. High gradient (>2%)
scode
1
2
3

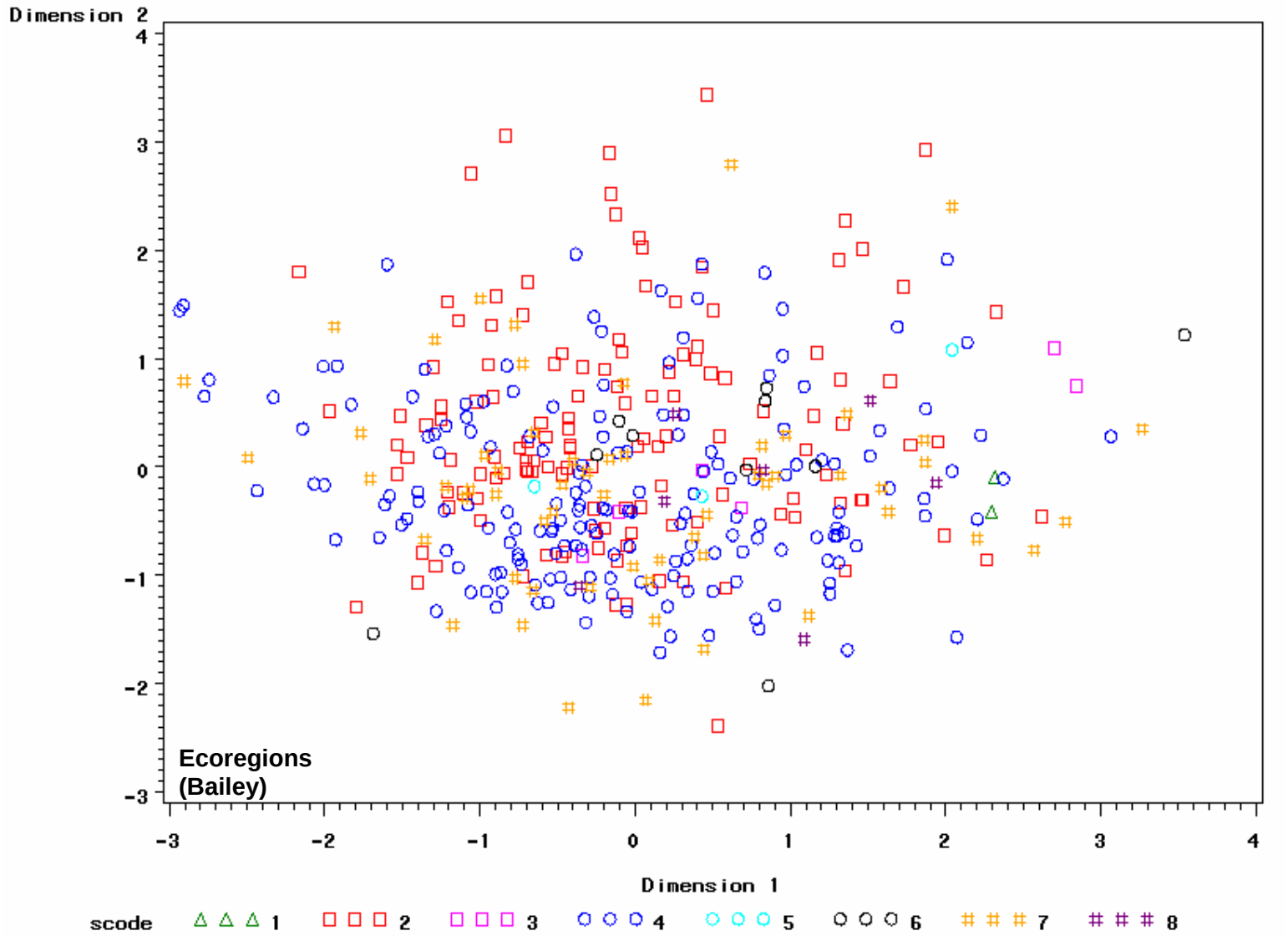

Dimension 2
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
4
Dimension 1
Major basin
1. Mid-Atlantic
2. Ohio River
scode
1
2


Dimension 2
4
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
Dimension 1
River basin
1. Allegheny
2. Lower Delaware
3. Lower Susquehanna
4. Monongahela
5. Upper Ohio
6. Potomac
7. Upper Delaware
8. Upper Susquehanna
9. West Branch Susquehanna
scode
1
2
3
4
5
6
7
8
9

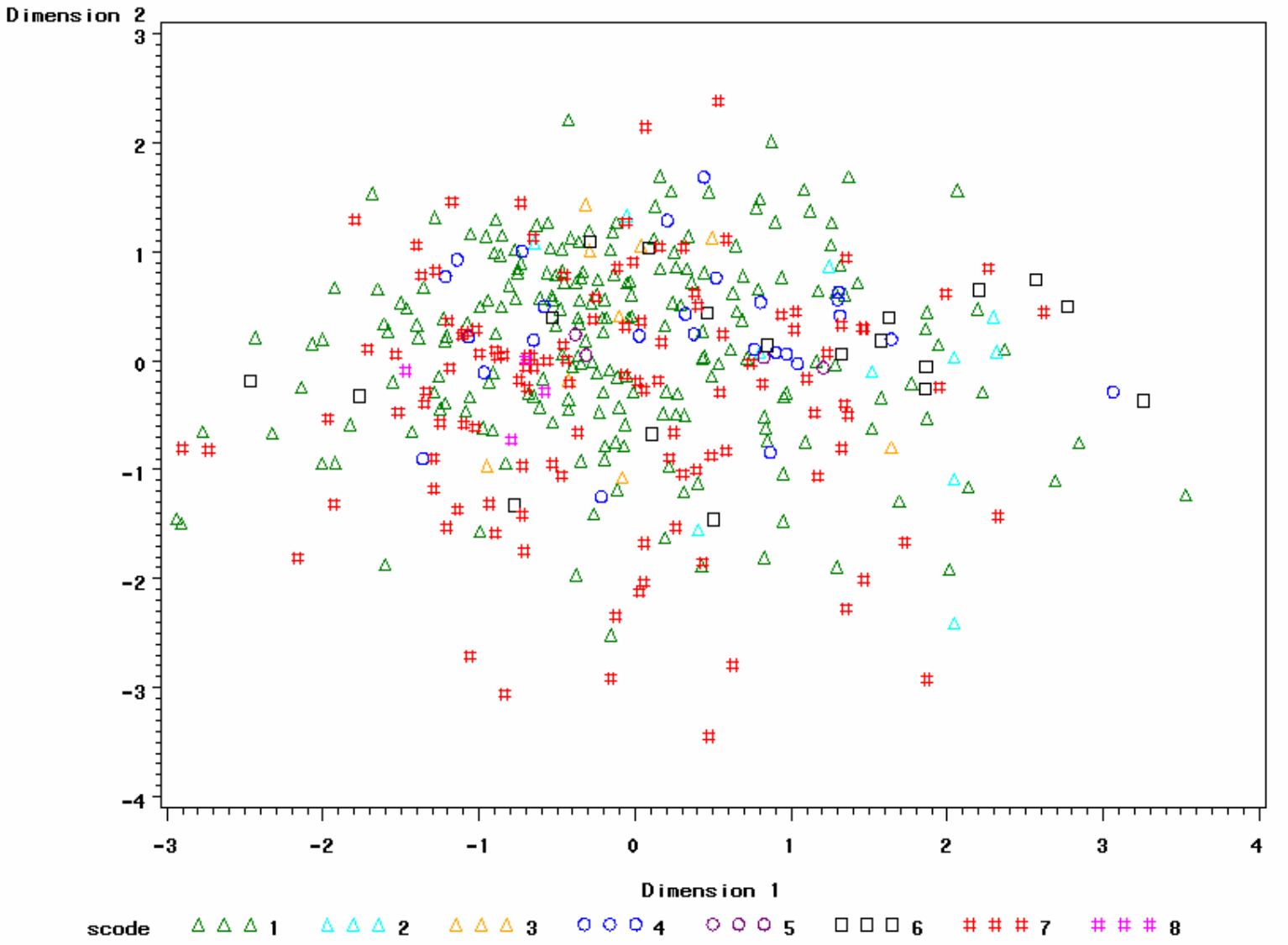

Dimension 2
4
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
Dimension 1
Bioregions
1. Mountains
2. Mountains / Piedmont
3. Mountains / Plateau
4. Piedmont
5. Piedmont / Mountains
6. Plateau
7. Plateau / Mountains
scode
1
2
3
4
5
6
7



1. Lower New England / Piedmont **2. Central Appalachian Forest**
3. Central App. Forest / High Allegheny Plateau **4. High Alleg. Plateau**
5. High Alleg. Plateau / Central App. Forest **6. High Alleg. Plateau / Western Alleg. Plateau**
7. Western Alleg. Plateau **8. Western Alleg. Plateau / High Alleg. Plateau**



1. Adirondack Forest… / Laurentian Mixed Forest
2. Central Appalachian Forest…
3. Central App. Forest… / Adirondack Forest…
4. Laurentian Mixed Forest
5. Laurentian Mixed Forest / Central App. Forest…
6. Laurentian Mixed Forest / Eastern Broadleaf Forest
7. Eastern Broadleaf Forest
8. Eastern Broadleaf Forest / Laurentian Mixed Forest



1. Atlantic Highlands **2. Atlantic Highlands / Mixed Wood Plains**
3. Atlantic Highlands / Appalachian Forest **4. Mixed Wood Plains**
5. Mixed Wood Plains / Atlantic Highlands **6. Southeastern USA Plains**
7. Appalachian Forest **8. Appalachian Forest / Atlantic Highlands**

Appendix D – Metric and Index Calculations

This appendix presents example metric calculations and proceeds step-by-step through the index development process using data from two samples at extremes of the condition spectrum: Lycoming Creek (173 square miles) in Lycoming County and the Youghiogheny River (433 square miles) in Somerset County. The taxa lists from the two sub-samples are below, followed by core metric calculations for each sample.

Lycoming Creek (Tier A)
20011119-0409-WQN

Taxa Name	Number of Individuals
Acentrella	1
Isonychia	4
Epeorus	6
Leucrocuta	1
Rhithrogena	9
Stenonema	8
Ephemerella	32
Serratella	1
Paraleptophlebia	4
Pteronarcys	1
Taeniopteryx	1
Leuctra	2
Agnetina	1
Paragnetina	1
Chimarra	1
Dolophilodes	1
Cheumatopsyche	25
Hydropsyche	22
Rhyacophila	16
Glossosoma	2
Brachycentrus	3
Micrasema	1
Apatania	2
Psilotreta	1
Psephenus	3
Optioservus	7
Atherix	1
Antocha	2
Hexatoma	5
Prosimulium	1
Chironomidae	49
Ancylidae	2
Oligochaeta	1

Youghiogheny River (Tier D)
20051005-0709-WQN

Taxa Name	Number of Individuals
Cheumatopsyche	31
Hydropsyche	3
Hydroptila	15
Simulium	30
Chironomidae	42
Hydrobiidae	3
Sphaeriidae	1
Oligochaeta	64
Tubificidae	2
Crangonyx	6
Caecidotea	7
Cladocera	1
Hydracarina	9

Total Taxa Richness

Lycoming Creek

= total number of taxa in a sub-sample

There are 33 taxa in this sub-sample.

Total Taxa Richness = 33

	Taxa Name	Number of Individuals
1	Acentrella	1
2	Isonychia	4
3	Epeorus	6
4	Leucrocuta	1
5	Rhithrogena	9
6	Stenonema	8
7	Ephemerella	32
8	Serratella	1
9	Paraleptophlebia	4
10	Pteronarcys	1
11	Taeniopteryx	1
12	Leuctra	2
13	Agnetina	1
14	Paragnetina	1
15	Chimarra	1
16	Dolophilodes	1
17	Cheumatopsyche	25
18	Hydropsyche	22
19	Rhyacophila	16
20	Glossosoma	2
21	Brachycentrus	3
22	Micrasema	1
23	Apatania	2
24	Psilotreta	1
25	Psephenus	3
26	Optioservus	7
27	Atherix	1
28	Antocha	2
29	Hexatoma	5
30	Prosimulium	1
31	Chironomidae	49
32	Ancylidae	2
33	Oligochaeta	1

Total Taxa Richness

Youghiogheny River

= total number of taxa in a sub-sample

There are 13 taxa in this sub-sample.

Total Taxa Richness = 13

	Taxa Name	Number of Individuals
1	Cheumatopsyche	31
2	Hydropsyche	3
3	Hydroptila	15
4	Simulium	30
5	Chironomidae	42
6	Hydrobiidae	3
7	Sphaeriidae	1
8	Oligochaeta	64
9	Tubificidae	2
10	Crangonyx	6
11	Caecidotea	7
12	Cladocera	1
13	Hydracarina	9

EPT Taxa Richness (PTV 0 – 4)

Lycoming Creek

= number of taxa belonging to the insect orders Ephemeroptera, Plecoptera, or Trichoptera with pollution tolerance values ≤ 4 in a sub-sample

There are 9 Ephemeroptera taxa (PTV ≤ 4) in this sub-sample.

Acentrella Isonychia Epeorus Leucrocuta
Rhithrogena Stenonema Ephemerella
Serratella Paraleptophlebia

There are 5 Plecoptera taxa (PTV ≤ 4) in this sub-sample.

Pteronarcys Taeniopteryx Leuctra
Agnetina Paragnetina

There are 8 Trichoptera taxa (PTV ≤ 4) in this sub-sample.

Chimarra Dolophilodes Rhyacophila
Glossosoma Brachycentrus Micrasema
Apatania Psilotreta

EPT Taxa Richness (PTV 0 – 4) = 9 + 5 + 8

EPT Taxa Richness (PTV 0 – 4) = 22

Taxa Name	Number of Individuals	Pollution Tolerance Value
Acentrella	1	4
Isonychia	4	3
Epeorus	6	0
Leucrocuta	1	1
Rhithrogena	9	0
Stenonema	8	3
Ephemerella	32	1
Serratella	1	2
Paraleptophlebia	4	1
Pteronarcys	1	0
Taeniopteryx	1	2
Leuctra	2	0
Agnetina	1	2
Paragnetina	1	1
Chimarra	1	4
Dolophilodes	1	0
Cheumatopsyche	25	6
Hydropsyche	22	5
Rhyacophila	16	1
Glossosoma	2	0
Brachycentrus	3	1
Micrasema	1	2
Apatania	2	3
Psilotreta	1	0
Psephenus	3	4
Optioservus	7	4
Atherix	1	2
Antocha	2	3
Hexatoma	5	2
Prosimulium	1	2
Chironomidae	49	6
Ancylidae	2	7
Oligochaeta	1	10

EPT Taxa Richness

Youghiogheny River

= number of taxa belonging to the insect orders Ephemeroptera, Plecoptera, or Trichoptera with pollution tolerance values ≤ 4 in a sub-sample

There are 0 Ephemeroptera taxa (PTV ≤ 4) in this sub-sample.

There are 0 Plecoptera taxa (PTV ≤ 4) in this sub-sample.

There are 0 Trichoptera taxa (PTV ≤ 4) in this sub-sample.

EPT Taxa Richness (PTV 0 – 4) = 0 + 0 + 0

EPT Taxa Richness (PTV 0 – 4) = 0

Taxa Name	Number of Individuals	Pollution Tolerance Value
Cheumatopsyche	31	6
Hydropsyche	3	5
Hydroptila	15	6
Simulium	30	6
Chironomidae	42	6
Hydrobiidae	3	8
Sphaeriidae	1	8
Oligochaeta	64	10
Tubificidae	2	10
Crangonyx	6	4
Caecidotea	7	6
Cladocera	1	5
Hydracarina	9	7

Beck's Index, version 3

Lycoming Creek

$= 3(n_{taxaHILS0}) + 2(n_{taxaHILS1}) + 1(n_{taxaHILS2})$

where $n_{taxaHILSi}$ = the number of taxa in a sub-sample with a pollution tolerance value (PTV) of i

There are 7 taxa in this sub-sample with PTV = 0.
There are 6 taxa in this sub-sample with PTV = 1.
There are 7 taxa in this sub-sample with PTV = 2.

Beck's Index, version 3 = 3(7) + 2(6) + 1(7)
Beck's Index, version 3 = 21 + 12 + 7
Beck's Index, version 3 = 40

Taxa Name	Number of Individuals	Pollution Tolerance Value
Acentrella	1	4
Isonychia	4	3
Epeorus	6	0
Leucrocuta	1	1
Rhithrogena	9	0
Stenonema	8	3
Ephemerella	32	1
Serratella	1	2
Paraleptophlebia	4	1
Pteronarcys	1	0
Taeniopteryx	1	2
Leuctra	2	0
Agnetina	1	2
Paragnetina	1	1
Chimarra	1	4
Dolophilodes	1	0
Cheumatopsyche	25	6
Hydropsyche	22	5
Rhyacophila	16	1
Glossosoma	2	0
Brachycentrus	3	1
Micrasema	1	2
Apatania	2	3
Psilotreta	1	0
Psephenus	3	4
Optioservus	7	4
Atherix	1	2
Antocha	2	3
Hexatoma	5	2
Prosimulium	1	2
Chironomidae	49	6
Ancylidae	2	7
Oligochaeta	1	10

Beck's Index, version 3

Youghiogheny River

$= 3(n_{taxaHILS0}) + 2(n_{taxaHILS1}) + 1(n_{taxaHILS2})$

where $n_{taxaHILSi}$ = the number of taxa in a sub-sample with a pollution tolerance value (PTV) of i

There are 0 taxa in this sub-sample with PTV = 0.
There are 0 taxa in this sub-sample with PTV = 1.
There are 0 taxa in this sub-sample with PTV = 2.

Beck's Index, version 3 = 3(0) + 2(0) + 1(0)
Beck's Index, version 3 = 0 + 0 + 0
Beck's Index, version 3 = 0

Taxa Name	Number of Individuals	Pollution Tolerance Value
Cheumatopsyche	31	6
Hydropsyche	3	5
Hydroptila	15	6
Simulium	30	6
Chironomidae	42	6
Hydrobiidae	3	8
Sphaeriidae	1	8
Oligochaeta	64	10
Tubificidae	2	10
Crangonyx	6	4
Caecidotea	7	6
Cladocera	1	5
Hydracarina	9	7

Hilsenhoff Biotic Index

Lycoming Creek

$$= \sum_{i=0}^{10} [(i * n_{indvPTVi})] / N$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with pollution tolerance value (PTV) of i and N = the total number of individuals in a sub-sample

There are 22 individuals with PTV = 0
There are 57 individuals with PTV = 1
There are 11 individuals with PTV = 2
There are 16 individuals with PTV = 3
There are 12 individuals with PTV = 4
There are 22 individuals with PTV = 5
There are 74 individuals with PTV = 6
There are 2 individuals with PTV = 7
There are 0 individuals with PTV = 8 or 9
There is 1 individual with PTV = 10.

There are a total of 217 individuals in the sub-sample.

Hilsenhoff Biotic Index =

[(0 * 22) + (1 * 57) + (2 * 11) +
(3 * 16) + (4 * 12) + (5 * 22) +
(6 * 74) + (7 * 2) + (8 * 0) +
(9 * 0) + (10 * 1)] / 217

Hilsenhoff Biotic Index = 3.47

Taxa Name	Number of Individuals	Pollution Tolerance Value
Acentrella	1	4
Isonychia	4	3
Epeorus	6	0
Leucrocuta	1	1
Rhithrogena	9	0
Stenonema	8	3
Ephemerella	32	1
Serratella	1	2
Paraleptophlebia	4	1
Pteronarcys	1	0
Taeniopteryx	1	2
Leuctra	2	0
Agnetina	1	2
Paragnetina	1	1
Chimarra	1	4
Dolophilodes	1	0
Cheumatopsyche	25	6
Hydropsyche	22	5
Rhyacophila	16	1
Glossosoma	2	0
Brachycentrus	3	1
Micrasema	1	2
Apatania	2	3
Psilotreta	1	0
Psephenus	3	4
Optioservus	7	4
Atherix	1	2
Antocha	2	3
Hexatoma	5	2
Prosimulium	1	2
Chironomidae	49	6
Ancylidae	2	7
Oligochaeta	1	10

Hilsenhoff Biotic Index

Youghiogheny River

$$= \sum_{i=0}^{10} [(i * n_{indvPTVi})] / N$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with pollution tolerance value (PTV) of i and N = the total number of individuals in a sub-sample

There are 0 individuals with PTV = 0, 1, 2 or 3
There are 6 individuals with PTV = 4
There are 4 individuals with PTV = 5
There are 125 individuals with PTV = 6
There are 9 individuals with PTV = 7
There are 4 individuals with PTV = 8
There are 0 individuals with PTV = 9
There are 66 individuals with PTV = 10.

There are a total of 214 individuals in the sub-sample.

Hilsenhoff Biotic Index =

[(0 * 0) + (1 * 0) + (2 * 0) +
(3 * 0) + (4 * 6) + (5 * 4) +
(6 * 125) + (7 * 9) + (8 * 4) +
(9 * 0) + (10 * 66)] / 214

Hilsenhoff Biotic Index = 7.24

Taxa Name	Number of Individuals	Pollution Tolerance Value
Cheumatopsyche	31	6
Hydropsyche	3	5
Hydroptila	15	6
Simulium	30	6
Chironomidae	42	6
Hydrobiidae	3	8
Sphaeriidae	1	8
Oligochaeta	64	10
Tubificidae	2	10
Crangonyx	6	4
Caecidotea	7	6
Cladocera	1	5
Hydracarina	9	7

Shannon Diversity Index

Lycoming Creek

$$= [- \sum_{i=1}^{Rich} (n_i / N) \ln (n_i / N)]$$

where n_i = the number of individuals in each taxa (relative abundance); N = the total number of individuals in a sub-sample; and Rich = the total number of taxa in a sub-sample (total taxa richness)

There are 33 taxa in this sub-sample. The numbers of individuals in each taxa are listed in the table to the right. There are a total of 217 individuals in the sub-sample.

Shannon Diversity Index =

– (1 / 217) ln (1 / 217) +
(4 / 217) ln (4 / 217) +
(6 / 217) ln (6 / 217) +
(1 / 217) ln (1 / 217) +
(9 / 217) ln (9 / 217) +
(8 / 217) ln (8 / 217) +
(32 / 217) ln (32 / 217) +
… *(do this for all 33 taxa)* …
(1 / 217) ln (1 / 217)

Shannon Diversity Index = 2.67

Taxa Name	Number of Individuals
Acentrella	1
Isonychia	4
Epeorus	6
Leucrocuta	1
Rhithrogena	9
Stenonema	8
Ephemerella	32
Serratella	1
Paraleptophlebia	4
Pteronarcys	1
Taeniopteryx	1
Leuctra	2
Agnetina	1
Paragnetina	1
Chimarra	1
Dolophilodes	1
Cheumatopsyche	25
Hydropsyche	22
Rhyacophila	16
Glossosoma	2
Brachycentrus	3
Micrasema	1
Apatania	2
Psilotreta	1
Psephenus	3
Optioservus	7
Atherix	1
Antocha	2
Hexatoma	5
Prosimulium	1
Chironomidae	49
Ancylidae	2
Oligochaeta	1

Shannon Diversity Index

Youghiogheny River

$$= [- \sum_{i=1}^{Rich} (n_i / N) \ln (n_i / N)]$$

where n_i = the number of individuals in each taxa (relative abundance); N = the total number of individuals in a sub-sample; and Rich = the total number of taxa in a sub-sample (total taxa richness)

There are 13 taxa in this sub-sample. The numbers of individuals in each taxa are listed in the table to the right. There are a total of 214 individuals in the sub-sample.

Taxa Name	Number of Individuals
Cheumatopsyche	31
Hydropsyche	3
Hydroptila	15
Simulium	30
Chironomidae	42
Hydrobiidae	3
Sphaeriidae	1
Oligochaeta	64
Tubificidae	2
Crangonyx	6
Caecidotea	7
Cladocera	1
Hydracarina	9

Shannon Diversity Index =

– (31 / 214) ln (31 / 214) +
(3 / 214) ln (3 / 214) +
(15 / 214) ln (15 / 214) +
(30 / 214) ln (30 / 214) +
(42 / 214) ln (42 / 214) +
(3 / 214) ln (3 / 214) +
(1 / 214) ln (1 / 214) +
… *(do this for all 13 taxa)* …
(9 / 214) ln (9 / 214)

Shannon Diversity Index = 1.98

Percent Sensitive (PTV 0 – 3) Individuals

Lycoming Creek

$$= \left(\sum_{i=0}^{3} n_{indvPTVi} \right) / N * 100$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with pollution tolerance value (PTV) of i and N = the total number of individuals in a sub-sample

There are 22 individuals with PTV = 0
There are 57 individuals with PTV = 1
There are 11 individuals with PTV = 2
There are 16 individuals with PTV = 3

There are a total of 217 individuals in the sub-sample.

Percent Sensitive (PTV 0 – 3) Individuals =
(22 + 57 + 11 + 16) / 217 *100

Percent Sensitive (PTV 0 – 3) Individuals =
106 / 217 * 100

Percent Sensitive (PTV 0 – 3) Individuals = 48.8%

Taxa Name	Number of Individuals	Pollution Tolerance Value
Acentrella	1	4
Isonychia	4	3
Epeorus	6	0
Leucrocuta	1	1
Rhithrogena	9	0
Stenonema	8	3
Ephemerella	32	1
Serratella	1	2
Paraleptophlebia	4	1
Pteronarcys	1	0
Taeniopteryx	1	2
Leuctra	2	0
Agnetina	1	2
Paragnetina	1	1
Chimarra	1	4
Dolophilodes	1	0
Cheumatopsyche	25	6
Hydropsyche	22	5
Rhyacophila	16	1
Glossosoma	2	0
Brachycentrus	3	1
Micrasema	1	2
Apatania	2	3
Psilotreta	1	0
Psephenus	3	4
Optioservus	7	4
Atherix	1	2
Antocha	2	3
Hexatoma	5	2
Prosimulium	1	2
Chironomidae	49	6
Ancylidae	2	7
Oligochaeta	1	10

Percent Sensitive (PTV 0 – 3) Individuals

Youghiogheny River

$$= \left(\sum_{i=0}^{3} n_{indvPTVi} \right) / N * 100$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with pollution tolerance value (PTV) of i and N = the total number of individuals in a sub-sample

There are 0 individuals with PTV = 0, 1, 2 or 3

There are a total of 214 individuals in the sub-sample.

Percent Sensitive (PTV 0 – 3) Individuals =
(0 + 0 + 0 + 0) / 214 *100

Percent Sensitive (PTV 0 – 3) Individuals =
0 / 214 * 100

Percent Sensitive (PTV 0 – 3) Individuals = 0%

Taxa Name	Number of Individuals	Pollution Tolerance Value
Cheumatopsyche	31	6
Hydropsyche	3	5
Hydroptila	15	6
Simulium	30	6
Chironomidae	42	6
Hydrobiidae	3	8
Sphaeriidae	1	8
Oligochaeta	64	10
Tubificidae	2	10
Crangonyx	6	4
Caecidotea	7	6
Cladocera	1	5
Hydracarina	9	7

Metric Standardization and Index Scoring

The Hilsenhoff Biotic Index metric values are expected to increase in value with increasing anthropogenic stress and were standardized to the 5th percentile of metric scores for all samples using the following equation:

Standardized score (ranging from 0 to 1) = (10 – observed value) / (10 – 5th percentile value of all samples)

The other five core metrics values are expected to decrease in value with increasing anthropogenic stress and were standardized to the 95th percentile of metric scores for all samples using the following equation:

Standardized score (ranging from 0 to 1) = observed value / 95th percentile value of all samples

Table D1 lists the relevant percentile values (i.e., 5th or 95th) of all samples for each core metric.

Table D1. Values used to standardize core metrics

Metric	5th percentile value	95th percentile value
Total Taxa Richness	---	33
EPT Taxa Richness (PTV 0 – 4)	---	19
Beck's Index, version 3	---	38
Hilsenhoff Biotic Index	1.89	---
Shannon Diversity	---	2.86
Percent Sensitive Individuals (PTV 0 – 3)	---	84.5

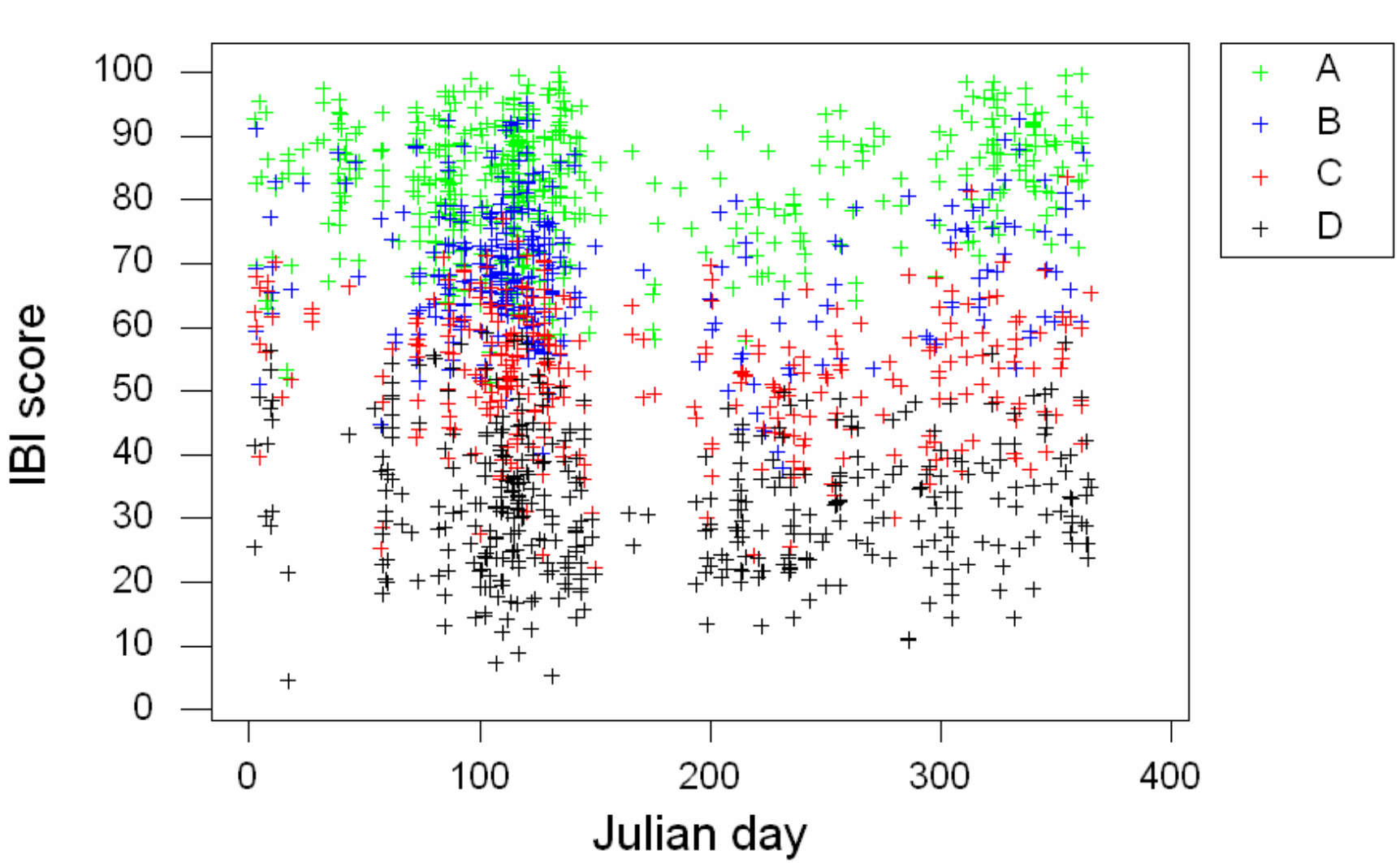
Table D2 and Table D3 show the standardization and index scoring calculations for the two samples discussed above.

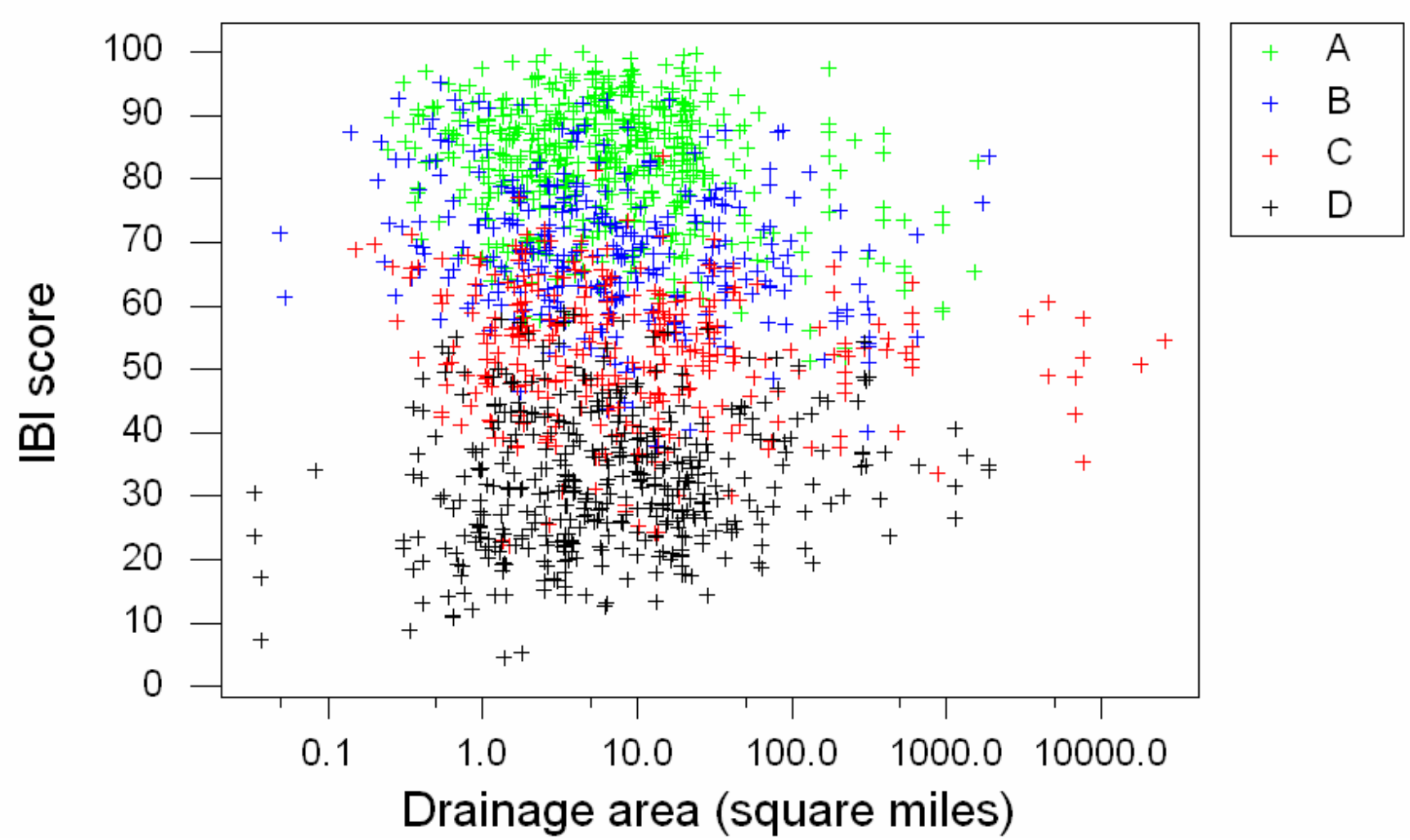
Table D2. Standardization and index calculations for the Lycoming Creek sample

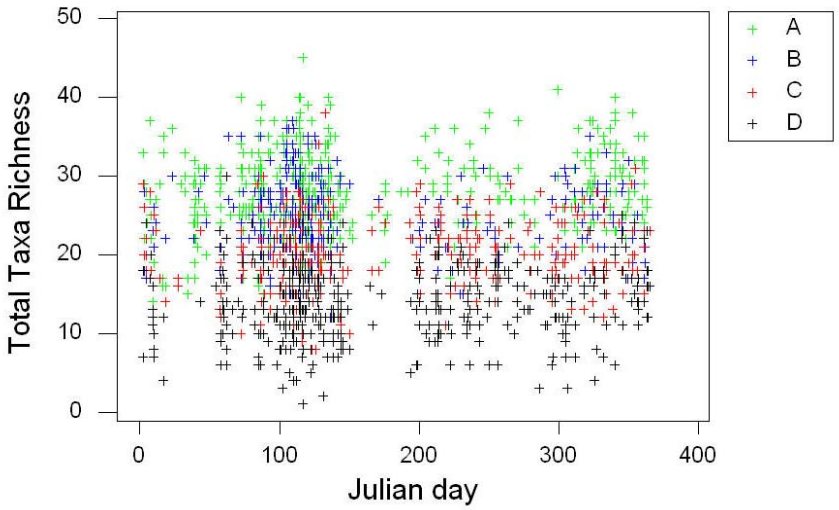
Metric	Standardization Equation	Observed Metric Value	Standardized Metric Score	Adjusted Standardized Metric Score Maximum = 1.000
Total Taxa Richness	observed value / 33	33	1.000	1.000
EPT Taxa Richness	observed value / 19	22	1.158	1.000
Modified Beck's Index	observed value / 38	40	1.053	1.000
Hilsenhoff Biotic Index	(10 – observed value) / (10 – 1.89)	3.47	0.805	0.805
Shannon Diversity	observed value / 2.86	2.67	0.934	0.934
Percent Sensitive Individuals	observed value / 84.5	48.8	0.578	0.578
Average of adjusted standardized core metric scores * 100 = **IBI Score =**				**88.6**

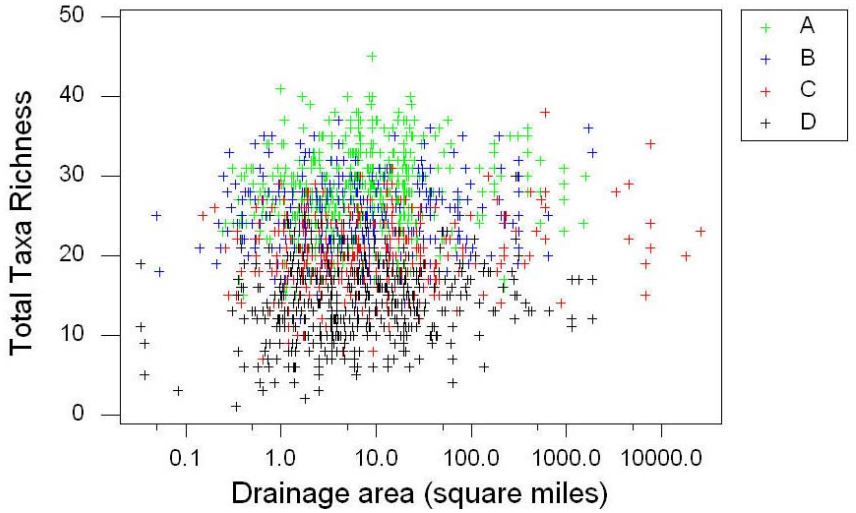
Table D3. Standardization and index calculations for the Youghiogheny RIver sample

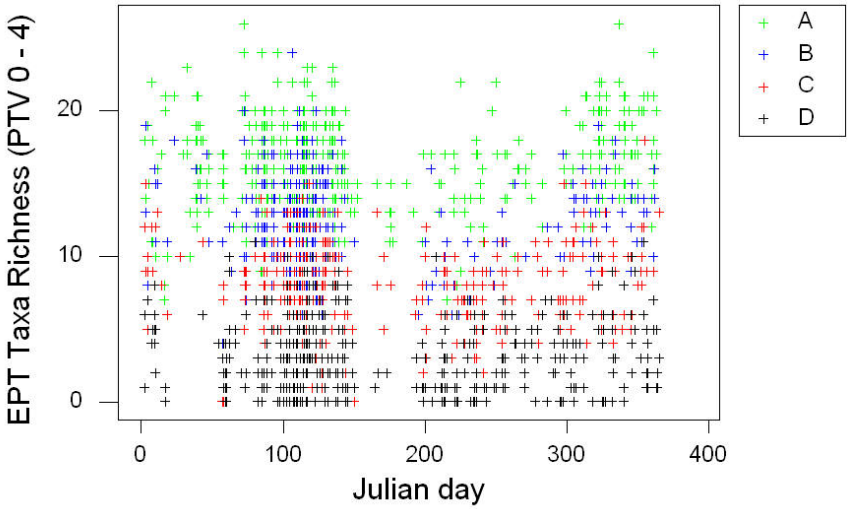
Metric	Standardization Equation	Observed Metric Value	Standardized Metric Score	Adjusted Standardized Metric Score Maximum = 1.000
Total Taxa Richness	observed value / 33	13	0.394	0.394
EPT Taxa Richness	observed value / 19	0	0.000	0.000
Modified Beck's Index	observed value / 38	0	0.000	0.000
Hilsenhoff Biotic Index	(10 – observed value) / (10 – 1.89)	7.24	0.340	0.340
Shannon Diversity	observed value / 2.86	1.98	0.692	0.692
Percent Sensitive Individuals	observed value / 84.5	0	0.000	0.000
Average of adjusted standardized core metric scores * 100 = **IBI Score =**				**23.8**

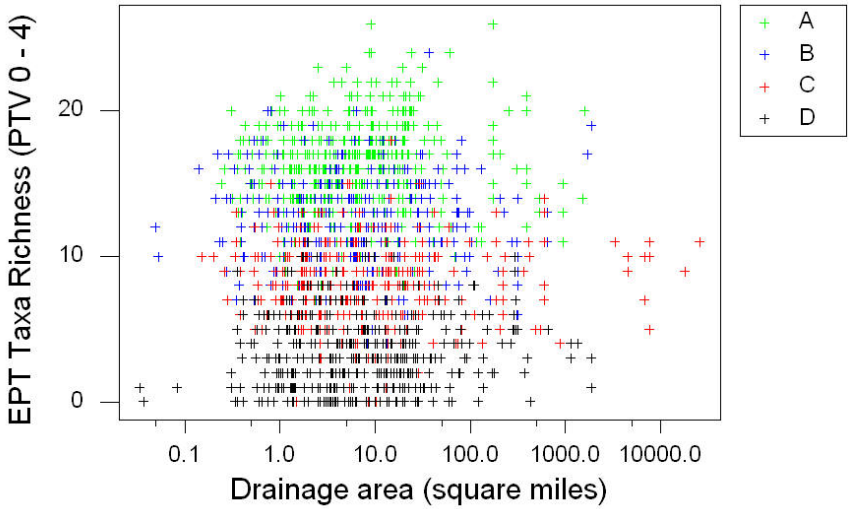

IBI score
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
Julian day
A
B
C
D

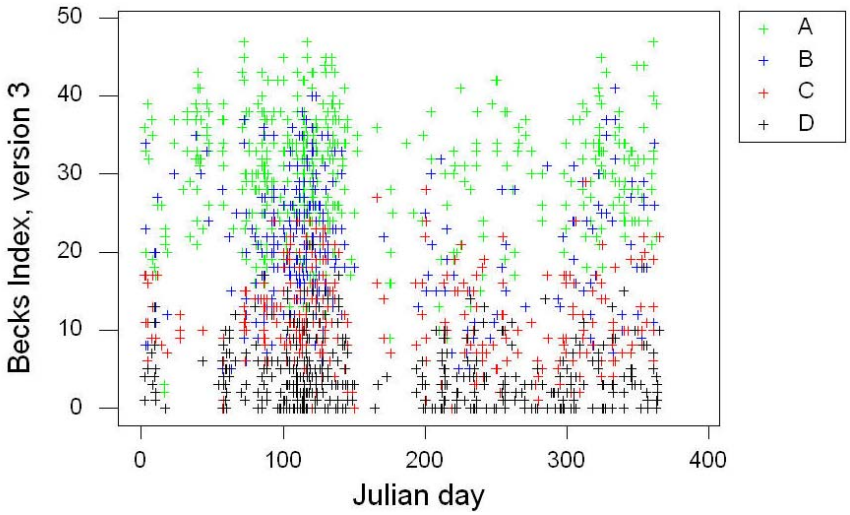

IBI score
100
90
80
70
60
50
40
30
20
10
0
0.1
1.0
10.0
100.0
1000.0
10000.0
Drainage area (square miles)
A
B
C
D

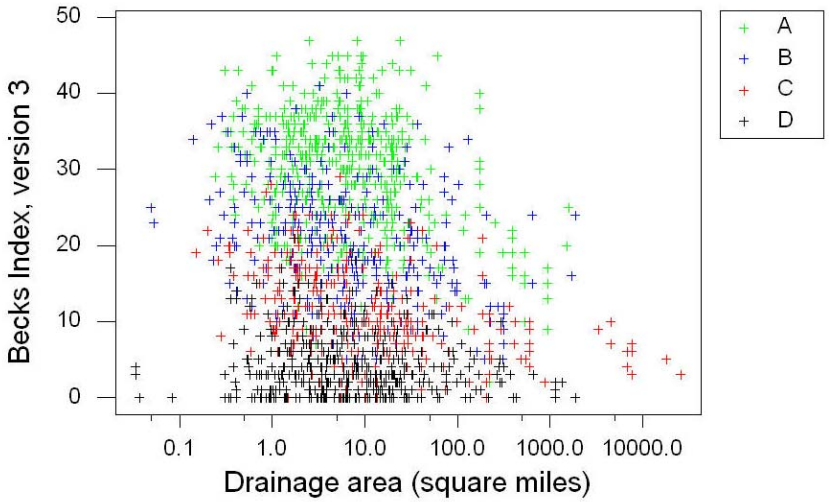

Total Taxa Richness
Julian day
A
B
C
D

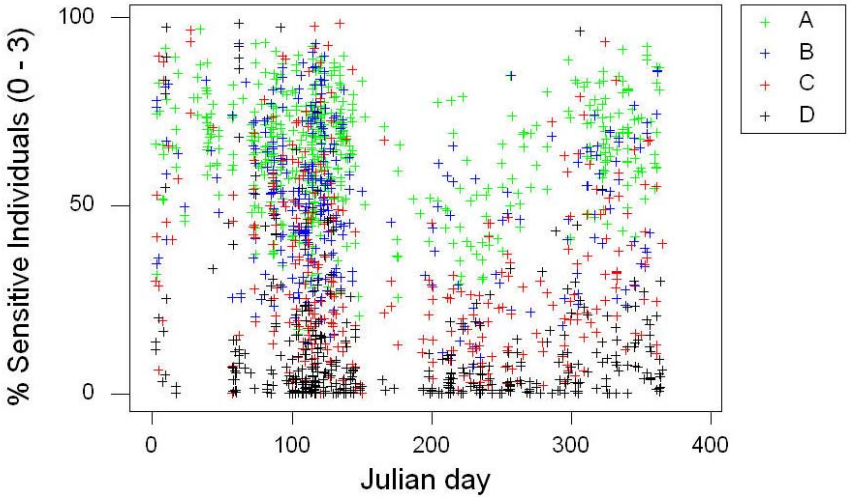

Total Taxa Richness
Drainage area (square miles)
A
B
C
D

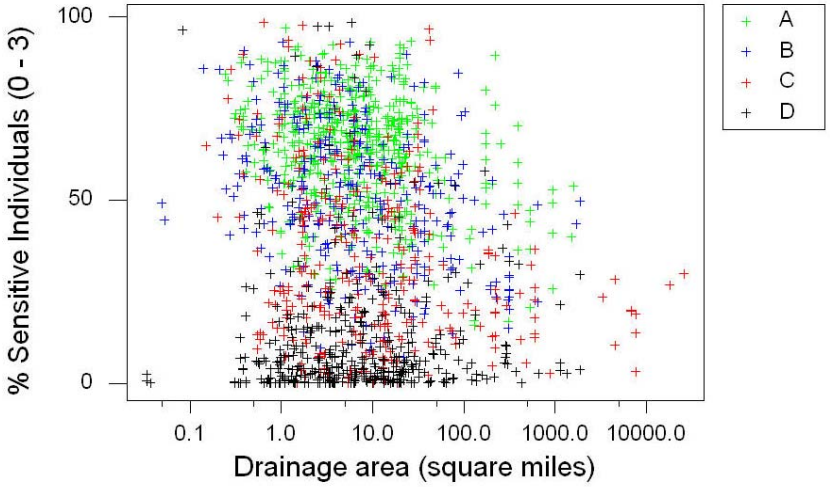

EPT Taxa Richness (PTV 0 - 4)
Julian day
A
B
C
D

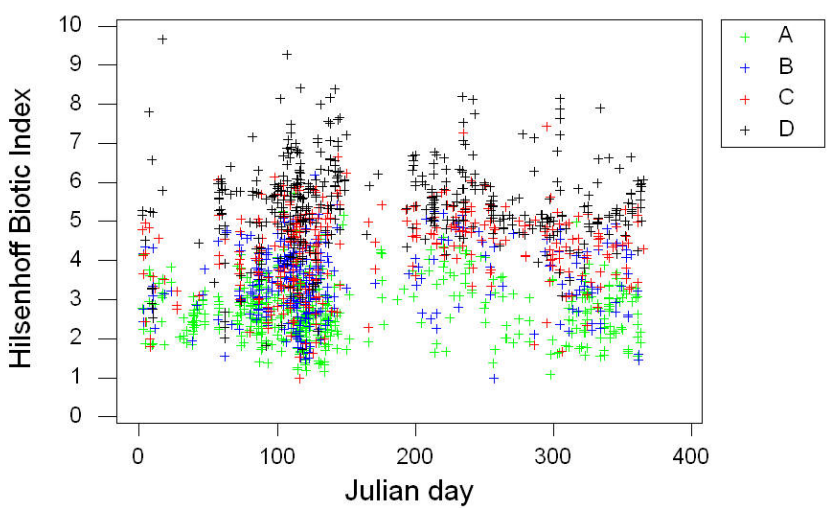

EPT Taxa Richness (PTV 0 - 4)
Drainage area (square miles)
A
B
C
D

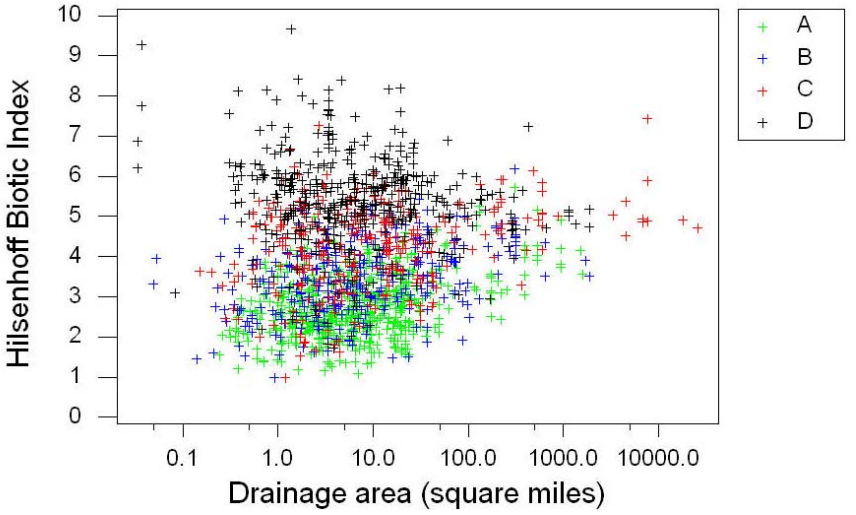

Becks Index, version 3
Julian day
A
B
C
D

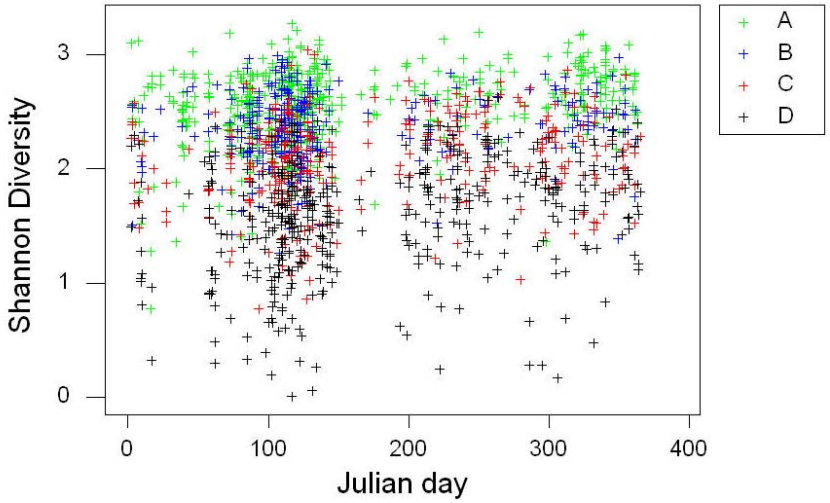

Becks Index, version 3
Drainage area (square miles)
A
B
C
D

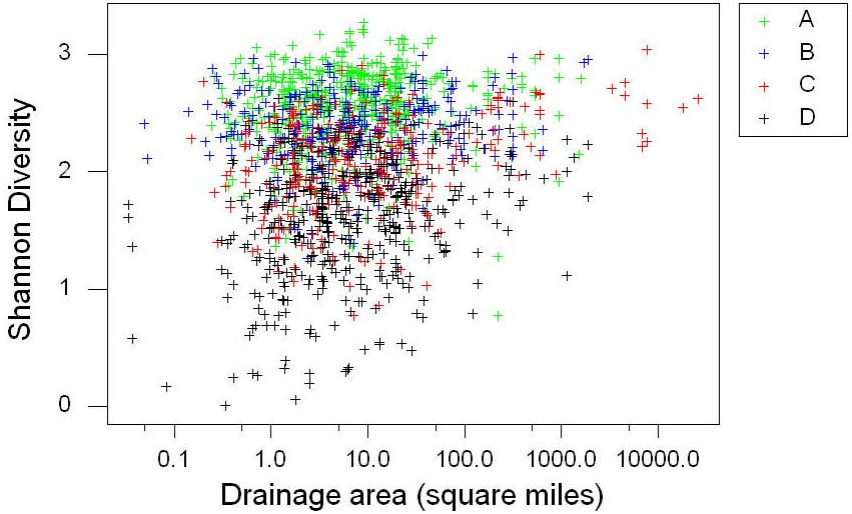

% Sensitive Individuals (0 - 3)
Julian day
A
B
C
D


% Sensitive Individuals (0 - 3)
Drainage area (square miles)
A
B
C
D


Hilsenhoff Biotic Index
Julian day
A
B
C
D


Hilsenhoff Biotic Index
Drainage area (square miles)
A
B
C
D


Shannon Diversity
Julian day
A
B
C
D


Shannon Diversity
Drainage area (square miles)
A
B
C
D

Appendix F – Master Taxa List

An taxonomically organized list of taxa names, pollution tolerance values and functional feeding groups for all aquatic benthic taxa recognized by DEP in Pennsylvania.

Taxa ID Level	Taxonomic Level	Pollution Tolerance Value	Functional Feeding Group
Insecta	**Class**		
Collembola	**Order**	9	CG
Onychiuridae	**Family**	9	CG
Onychiurus	Genus	9	CG
Poduridae	**Family**	9	CG
Podura	Genus	9	CG
Ephemeroptera	**Order**		
Ameletidae	**Family**	0	CG
Ameletus	Genus	0	CG
Siphlonuridae	**Family**	7	CG
Siphlonisca	Genus	7	PR
Siphlonurus	Genus	7	CG
Metretopodidae	**Family**	2	CG
Metrotopus	Genus	2	CG
Baetidae	**Family**	6	CG
Acentrella	Genus	4	SC
Acerpenna	Genus	6	CG
Baetis	Genus	6	CG
Barbaetis	Genus	6	CG
Callibaetis	Genus	9	CG
Centroptilum	Genus	2	CG
Cloeon	Genus	4	CG
Diphetor	Genus	6	CG
Fallceon	Genus	6	CG
Labiobaetis	Genus	2	SC
Procloeon	Genus	6	CG
Heterocloeon	Genus	2	SC
Plauditus	Genus	4	CG
Isonychiidae	**Family**	3	CG
Isonychia	Genus	3	CG
Heptageniidae	**Family**	3	SC
Epeorus	Genus	0	SC
Heptagenia	Genus	4	SC
Leucrocuta	Genus	1	SC
Nixe	Genus	2	SC
Rhithrogena	Genus	0	CG
Stenacron	Genus	4	SC
Stenonema	Genus	3	SC
Cinygmula	Genus	1	CG
Arthropleidae	**Family**	3	SC
Arthroplea	Genus	3	SC
Ephemerellidae	**Family**	2	CG
Attenella	Genus	2	SC
Drunella	Genus	1	SC
Ephemerella	Genus	1	CG
Eurylophella	Genus	4	SC
Serratella	Genus	2	CG
Timpanoga	Genus	2	CG
Neoephemeridae	**Family**	3	CG
Neoephemera	Genus	3	CG
Caenidae	**Family**	7	CG
Brachycercus	Genus	3	CG
Caenis	Genus	7	CG
Baetiscidae	**Family**	3	CG
Baetisca	Genus	4	CG
Leptophlebiidae	**Family**	4	CG
Choroterpes	Genus	2	CG
Habrophlebia	Genus	4	CG
Habrophlebiodes	Genus	6	SC
Leptophlebia	Genus	4	CG
Paraleptophlebia	Genus	1	CG
Potamanthidae	**Family**	4	CG
Anthopotamus	Genus	4	CG
Ephemeridae	**Family**	4	CG
Ephemera	Genus	2	CG
Hexagenia	Genus	6	CG
Litobrancha	Genus	6	CG
Pentagenia	Genus	4	CG
Polymitarcyidae	**Family**	2	CG
Ephoron	Genus	2	CG
Tricorythidae	**Family**	4	CG
Tricorythodes	Genus	4	CG
Leptohyphes	Genus	4	CG
Odonata	**Order**		PR
Petaluridae	**Family**	5	PR
Tachopteryx	Genus	5	PR
Gomphidae	**Family**	4	PR
Aphylla	Genus	4	PR
Arigomphus	Genus	4	PR
Dromogomphus	Genus	4	PR
Erpetogomphus	Genus	5	PR
Gomphus	Genus	5	PR
Hagenius	Genus	3	PR

Lanthus	Genus	5	PR
Ophiogomphus	Genus	1	PR
Progomphus	Genus	5	PR
Stylogomphus	Genus	4	PR
Stylurus	Genus	4	PR
Aeshnidae	**Family**	3	PR
Aeshna	Genus	5	PR
Anax	Genus	5	PR
Basiaeschna	Genus	2	PR
Boyeria	Genus	2	PR
Epiaeschna	Genus	2	PR
Gomphaeschna	Genus	2	PR
Nasiaeschna	Genus	2	PR
Cordulegastridae	**Family**	3	PR
Cordulegaster	Genus	3	PR
Corduliidae	**Family**	5	PR
Didymops	Genus	4	PR
Cordulia	Genus	4	PR
Dorocordulia	Genus	4	PR
Epitheca	Genus	4	PR
Helocordulia	Genus	2	PR
Somatochlora	Genus	1	PR
Williamsonia	Genus	4	PR
Macromia	Genus	2	PR
Neurocordulia	Genus	3	PR
Libellulidae	**Family**	9	PR
Celithemis	Genus	2	PR
Erythemis	Genus	5	PR
Erythrodiplax	Genus	5	PR
Ladona	Genus	6	PR
Leucorrhinia	Genus	6	PR
Libellula	Genus	8	PR
Nannothemis	Genus	6	PR
Pachydiplax	Genus	8	PR
Pantala	Genus	7	PR
Perithemis	Genus	4	PR
Plathemis	Genus	3	PR
Sympetrum	Genus	4	PR
Tramea	Genus	4	PR
Calopterygidae	**Family**	5	PR
Calopteryx	Genus	6	PR
Hetaerina	Genus	6	PR
Lestes	Genus	9	PR
Coenagrionidae	**Family**	8	PR
Amphiagrion	Genus	5	PR
Argia	Genus	6	PR
Chromagrion	Genus	4	PR
Coenagrion	Genus	8	PR
Enallagma	Genus	8	PR
Ischnura	Genus	9	PR
Nehalennia	Genus	7	PR
Plecoptera	**Order**		PR
Pteronarcyidae	**Family**	0	SH
Pteronarcys	Genus	0	SH
Peltoperlidae	**Family**	2	SH
Peltoperla	Genus	2	SH
Tallaperla	Genus	0	SH
Viehoperla	Genus	2	SH
Taeniopterygidae	**Family**	2	SH
Taeniopteryx	Genus	2	SH
Bolotoperla	Genus	2	SH
Oemopteryx	Genus	3	SH
Strophopteryx	Genus	3	SH
Taenionema	Genus	3	SH
Nemouridae	**Family**	2	SH
Amphinemura	Genus	3	SH
Ostrocerca	Genus	2	SH
Paranemoura	Genus	2	SH
Podmosta	Genus	2	SH
Prostoia	Genus	2	SH
Shipsa	Genus	2	SH
Soyedina	Genus	0	SH
Zapada	Genus	2	SH
Nemoura	Genus	1	SH
Leuctridae	**Family**	0	SH
Megaleuctra	Genus	0	SH
Leuctra	Genus	0	SH
Paraleuctra	Genus	0	SH
Zealeuctra	Genus	0	SH
Capniidae	**Family**	3	SH
Allocapnia	Genus	3	SH
Capnia	Genus	1	SH
Nemocapnia	Genus	1	SH
Paracapnia	Genus	1	SH
Utacapnia	Genus	1	SH
Capnura	Genus	1	SH
Perlidae	**Family**	3	PR
Agnetina	Genus	2	PR
Hansonoperla	Genus	3	PR
Neoperla	Genus	3	PR
Paragnetina	Genus	1	PR
Acroneuria	Genus	0	PR
Attaneuria	Genus	3	PR
Eccoptura	Genus	2	PR
Perlesta	Genus	4	PR
Beloneuria	Genus	3	PR
Perlinella	Genus	2	PR
Perlodidae	**Family**	2	PR
Cultus	Genus	2	PR
Diploperla	Genus	2	PR
Diura	Genus	2	PR
Helopicus	Genus	2	PR
Hydroperla	Genus	1	PR
Isogenoides	Genus	0	PR
Malirekus	Genus	2	PR
Oconoperla	Genus	2	PR
Remenus	Genus	2	PR
Yugus	Genus	2	PR
Clioperla	Genus	2	PR
Isoperla	Genus	2	PR

Arcynopteryx	Genus	2	PR
Chloroperlidae	**Family**	0	PR
Utaperla	Genus	0	PR
Alloperla	Genus	0	CG
Haploperla	Genus	0	PR
Rasvena	Genus	0	PR
Suwallia	Genus	0	CG
Sweltsa	Genus	0	PR
Hemiptera	**Order**		
Hydrometridae	**Family**	9	PR
Hydrometridae	Genus	9	PR
Veliidae	**Family**	8	PR
Microvelia	Genus	9	PR
Rhagovelia	Genus	9	PR
Steinovelia	Genus	9	PR
Ceratocombidae	**Family**	9	PR
Ceratocombus	Genus	9	PR
Gerridae	**Family**	9	PR
Aquarius	Genus	9	PR
Gerris	Genus	9	PR
Halobates	Genus	9	PR
Rheumatobates	Genus	9	PR
Metrobates	Genus	9	PR
Trepobates	Genus	9	PR
Limnoporus	Genus	9	PR
Belostomatidae	**Family**	9	PR
Belostoma	Genus	9	PR
Lethocerus	Genus	9	PR
Nepidae	**Family**	8	PR
Nepa	Genus	8	PR
Ranatra	Genus	8	PR
Pleidae	**Family**	8	PR
Neoplea	Genus	8	PR
Naucoridae	**Family**	8	PR
Pelocoris	Genus	8	PR
Corixidae	**Family**	8	PR
Hesperocorixa	Genus	5	PR
Palmacorixa	Genus	8	PR
Ramphocorixa	Genus	8	PR
Sigara	Genus	8	PR
Trichocorixa	Genus	8	PR
Notonectidae	**Family**	8	PR
Buenoa	Genus	8	PR
Notonecta	Genus	8	PR
Mesoveliidae	**Family**	9	PR
Mesovelia	Genus	9	PR
Hebridae	**Family**	8	PR
Hebrus	Genus	8	PR
Merragata	Genus	8	PR
Saldidae	**Family**	8	PR
Micracanthia	Genus	8	PR
Pentacora	Genus	8	PR
Salda	Genus	8	PR
Saldula	Genus	8	PR
Gelastocoridae	**Family**	8	PR

Gelastocoris	Genus	8	PR
Ochteridae	**Family**	8	PR
Ochterus	Genus	8	PR
Megaloptera	**Order**	8	PR
Sialidae	**Family**	6	PR
Sialis	Genus	6	PR
Corydalidae	**Family**	3	PR
Corydalus	Genus	4	PR
Chauliodes	Genus	4	PR
Neohermes	Genus	2	PR
Nigronia	Genus	2	PR
Neuroptera	**Order**	3	PR
Sisyridae	**Family**	1	PI
Climacia	Genus	1	PI
Sisyra	Genus	1	PI
Trichoptera	**Order**		
Philopotamidae	**Family**	3	FC
Chimarra	Genus	4	FC
Dolophilodes	Genus	0	FC
Wormaldia	Genus	0	FC
Psychomyiidae	**Family**	2	CG
Lype	Genus	2	CG
Psychomyia	Genus	2	CG
Polycentropodidae	**Family**	6	FC
Cernotina	Genus	6	PR
Cyrnellus	Genus	8	FC
Neureclipsis	Genus	7	FC
Paranyctiophylax	Genus	7	FC
Polycentropus	Genus	6	FC
Phylocentropus	Genus	5	FC
Nyctiophylax	Genus	5	PR
Hydropsychidae	**Family**	5	FC
Arctopsyche	Genus	1	FC
Parapsyche	Genus	0	FC
Diplectrona	Genus	0	FC
Homoplectra	Genus	5	FC
Ceratopsyche	Genus	5	FC
Cheumatopsyche	Genus	6	FC
Hydropsyche	Genus	5	FC
Potamyia	Genus	5	FC
Macrostemum	Genus	3	FC
Rhyacophilidae	**Family**	1	SC
Rhyacophila	Genus	1	PR
Glossosomatidae	**Family**	0	SC
Glossosoma	Genus	0	SC
Agapetus	Genus	0	SC
Culoptila	Genus	1	SC
Protoptila	Genus	1	SC
Hydroptilidae	**Family**	4	PI
Palaeagapetus	Genus	1	SH
Agraylea	Genus	8	CG
Dibusa	Genus	4	SC
Hydroptila	Genus	6	SC
Ochrotrichia	Genus	4	SC

Oxyethira	Genus	3	CG
Stactobiella	Genus	2	SC
Leucotrichia	Genus	6	SC
Ithytrichia	Genus	6	SC
Orthotrichia	Genus	6	SH
Neotrichia	Genus	2	SC
Mayatrichia	Genus	4	SC
Phryganeidae	**Family**	4	SH
Agrypnia	Genus	7	SH
Banksiola	Genus	2	SH
Banksiola	Genus	2	SH
Fabria	Genus	4	SH
Hagenella	Genus	5	SH
Oligostomis	Genus	5	SH
Phryganea	Genus	8	SH
Ptilostomis	Genus	5	SH
Brachycentridae	**Family**	1	FC
Adicrophleps	Genus	2	SH
Brachycentrus	Genus	1	FC
Micrasema	Genus	2	SH
Lepidostomatidae	**Family**	1	SH
Lepidostoma	Genus	1	SH
Theliopsyche	Genus	1	SH
Limnephilidae	**Family**	4	SH
Ironoquia	Genus	3	SH
Onocosmoecus	Genus	3	SH
Apatania	Genus	3	SC
Pseudostenophylax	Genus	0	SH
Anabolia	Genus	5	SH
Arctopora	Genus	5	SH
Clostoeca	Genus	5	SH
Frenesia	Genus	4	SH
Hesperophylax	Genus	4	CG
Hydatophylax	Genus	2	SH
Leptophylax	Genus	2	SH
Limnephilus	Genus	3	SH
Philarctus	Genus	3	SH
Platycentropus	Genus	4	SH
Pycnopsyche	Genus	4	SH
Goera	Genus	0	SC
Madeophylax	Genus	4	SH
Glyphopsyche	Genus	3	SH
Uenoidae	**Family**	3	SC
Neophylax	Genus	3	SC
Beraeidae	**Family**	3	SC
Beraea	Genus	3	SC
Sericostomatidae	**Family**	3	SH
Agarodes	Genus	3	SH
Psilotreta	Genus	0	SC
Molannidae	**Family**	6	SC
Molanna	Genus	6	SC
Helicopsychidae	**Family**	3	SC
Helicopsyche	Genus	3	SC
Calamoceratidae	**Family**	5	SH
Heteroplectron	Genus	5	SH

Leptoceridae	**Family**	4	PR
Ceraclea	Genus	3	CG
Leptocerus	Genus	3	SH
Mystacides	Genus	4	CG
Nectopsyche	Genus	3	SH
Oecetis	Genus	8	PR
Setodes	Genus	2	CG
Triaenodes	Genus	6	SH
Odontoceridae	**Family**	0	SH
Lepidoptera	**Order**	5	SH
Pyralidae	**Family**	5	SH
Langessa	Genus	5	SH
Munroessa	Genus	5	SH
Neocataclysta	Genus	5	SH
Nymphula	Genus	7	SH
Nymphuliella	Genus	5	SH
Parapoynx	Genus	5	SH
Synclita	Genus	5	FC
Eoparargyractis	Genus	5	SH
Petrophila	Genus	5	SC
Acentria	Genus	5	SH
Schoenobius	Genus	5	SH
Chilo	Genus	5	SH
Acigona	Genus	5	SH
Ostrinia	Genus	5	SH
Nepticulidae	**Family**	5	SH
Stigmella	Genus	5	SH
Cosmopterigidae	**Family**	5	SH
Cosmopteryx	Genus	5	SH
Lymnaecia	Genus	5	SH
Noctuidae	**Family**	5	SH
Archanara	Genus	5	SH
Bellura	Genus	5	SH
Simyra	Genus	5	SH
Tortricidae	**Family**	5	SH
Archips	Genus	5	SH
Coleophoridae	**Family**	6	SH
Colephora	Genus	6	SH
Coleoptera	**Order**		
Gyrinidae	**Family**	4	PR
Dineutus	Genus	4	PR
Gyrinus	Genus	4	PR
Spanglerogyrus	Genus	4	PR
Haliplidae	**Family**	5	SH
Haliplus	Genus	5	SH
Peltodytes	Genus	5	SH
Dytiscidae	**Family**	5	PR
Acilius	Genus	5	PR
Agabetes	Genus	5	PR
Agabus	Genus	5	PR
Bidessonotus	Genus	5	PR
Brachyvatus	Genus	5	PR
Celina	Genus	5	PR
Copelatus	Genus	5	PR

Colymbetes	Genus	5	PR
Coptotomus	Genus	5	PR
Cybister	Genus	5	PR
Desmopachria	Genus	5	PR
Dytiscus	Genus	5	PR
Graphoderus	Genus	5	PR
Hydaticus	Genus	5	PR
Hydrovatus	Genus	5	PR
Hygrotus	Genus	5	PR
Ilybius	Genus	5	PR
Laccophilus	Genus	5	PR
Laccornis	Genus	5	PR
Liodessus	Genus	5	PR
Lioporius	Genus	5	PR
Matus	Genus	5	PR
Nebrioporus	Genus	5	PR
Oreodytes	Genus	5	PR
Rhantus	Genus	5	PR
Stictotarsus	Genus	5	PR
Uvarus	Genus	5	PR
Noteridae	**Family**	5	PR
Hydrocanthus	Genus	5	PR
Pronoterus	Genus	5	PR
Suphis	Genus	5	PR
Suphisellus	Genus	5	PR
Helophoridae	**Family**	5	SH
Helophorus	Genus	5	SH
Hydrochidae	**Family**	5	SH
Hydrochus	Genus	5	SH
Hydrophilidae	**Family**	5	PR
Anacaena	Genus	5	PR
Berosus	Genus	5	PR
Chaetarthria	Genus	5	PR
Crenitis	Genus	5	PR
Cymbiodyta	Genus	5	PR
Derallus	Genus	5	PR
Dibolocelus	Genus	5	PR
Enochrus	Genus	5	PR
Helochares	Genus	5	PR
Helocombus	Genus	5	PR
Hydrobius	Genus	5	PR
Hydrochara	Genus	5	PR
Hydrophilus	Genus	5	PR
Lacobius	Genus	5	PR
Paracymus	Genus	5	PR
Sperchopsis	Genus	5	PR
Tropisternus	Genus	5	PR
Staphylinidae	**Family**	5	PR
Bledius	Genus	5	PR
Carpelimus	Genus	5	PR
Psephidonus	Genus	5	PR
Thinobius	Genus	5	PR
Stenus	Genus	5	PR
Hydraenidae	**Family**	6	CG
Hydraena	Genus	6	CG
Limnebius	Genus	6	CG
Ochthebius	Genus	6	CG
Psephenidae	**Family**	4	SC
Eubrianax	Genus	4	SC
Psephenus	Genus	4	SC
Dicranopselaphus	Genus	4	SC
Ectopria	Genus	5	SC
Dryopidae	**Family**	5	SC
Dryops	Genus	5	SC
Helichus	Genus	5	SC
Scirtidae	**Family**	8	SC
Cyphon	Genus	8	SC
Elodes	Genus	8	SC
Flavohelodes	Genus	8	SC
Scirtes	Genus	8	SC
Elmidae	**Family**	5	CG
Ancyronyx	Genus	2	CG
Dubiraphia	Genus	6	SC
Gonielmis	Genus	5	SC
Macronychus	Genus	2	SC
Microcylloepus	Genus	2	SC
Optioservus	Genus	4	SC
Ordobrevia	Genus	5	SC
Oulimnius	Genus	5	SC
Promoresia	Genus	2	SC
Stenelmis	Genus	5	SC
Ptilodactylidae	**Family**	5	SH
Anchytarsus	Genus	5	SH
Lutrochidae	**Family**	6	UK
Lutrochus	Genus	6	UK
Chrysomelidae	**Family**	5	SH
Disonycha	Genus	5	SH
Donacia	Genus	5	SH
Hydrothassa	Genus	5	SH
Neohaemonia	Genus	5	SH
Prasocuris	Genus	5	SH
Pyrrhalta	Genus	5	SH
Curculionidae	**Family**	6	SH
Auleutes	Genus	6	SH
Bagous	Genus	6	SH
Brachybamus	Genus	6	SH
Euhrychiopsis	Genus	6	SH
Lissorhoptrus	Genus	6	SH
Listronotus	Genus	6	SH
Lixellus	Genus	6	SH
Lixus	Genus	6	SH
Notiodes	Genus	6	SH
Onychylis	Genus	6	SH
Perenthis	Genus	6	SH
Pelenomus	Genus	6	SH
Phytobius	Genus	6	SH
Stenopelmus	Genus	6	SH
Steremnius	Genus	6	SH
Tanysphyrus	Genus	6	SH
Histeridae	**Family**	5	SH

Hymenoptera	**Order**		
Pompilidae	**Family**	5	UK
Anoplius	Genus	5	UK
Scelionidae	**Family**	5	UK
Pseudanteris	Genus	5	UK
Telenomus	Genus	5	UK
Thoron	Genus	5	UK
Tiphodytes	Genus	5	UK
Diapriidae	**Family**	5	UK
Trichopria	Genus	5	UK
Ichneumonidae	**Family**	5	UK
Apsilops	Genus	5	UK
Cremastus	Genus	5	UK
Medophron	Genus	5	UK
Mesoleptus	Genus	5	UK
Phygadeuon	Genus	5	UK
Braconidae	**Family**	5	UK
Ademon	Genus	5	UK
Aphanta	Genus	5	UK
Asobara	Genus	5	UK
Bracon	Genus	5	UK
Chaenusa	Genus	5	UK
Chorebidella	Genus	5	UK
Chorebus	Genus	5	UK
Dacnusa	Genus	5	UK
Opius	Genus	5	UK
Phaenocarpa	Genus	5	UK
Mymaridae	**Family**	5	UK
Caraphractus	Genus	5	UK
Trichogrammatida	**Family**	5	UK
Hydrophylita	Genus	5	UK
Lathromeroidea	Genus	5	UK
Paracentrobia	Genus	5	UK
Trichogramma	Genus	5	UK
Eulophidae	**Family**	5	UK
Aprostocetus	Genus	5	UK
Mestocharis	Genus	5	UK
Tetrastichus	Genus	5	UK
Pteromalidae	**Family**	5	UK
Gyrinophagus	Genus	5	UK
Sisridivora	Genus	5	UK
Eucoilidae	**Family**	5	UK
Hexacola	Genus	5	UK
Diptera	**Order**		
Blephariceridae	**Family**	0	SC
Blepharicera	Genus	0	SC
Ceratopogonidae	**Family**	6	PR
Dasyhelea	Genus	6	CG
Atrichopogon	Genus	2	PR
Forcipomyia	Genus	6	SC
Alluaudomyia	Genus	6	PR
Bezzia	Genus	6	PR
Brachypogon	Genus	6	PR
Ceratopogon	Genus	6	PR

Clinohelea	Genus	6	PR
Culicoides	Genus	10	PR
Johannsenomyia	Genus	6	PR
Mallochohelea	Genus	6	PR
Monohelea	Genus	6	PR
Nilobezzia	Genus	6	PR
Palpomyia	Genus	6	PR
Probezzia	Genus	6	PR
Serromyia	Genus	6	PR
Sphaeromias	Genus	6	PR
Stilobezzia	Genus	6	PR
Leptoconops	Genus	6	PR
Chaoboridae	**Family**	8	PR
Chaoborus	Genus	8	PR
Mochlonyx	Genus	8	PR
Dixidae	**Family**	1	CG
Dixa	Genus	1	CG
Dixella	Genus	1	CG
Nymphomyiidae	**Family**	6	SC
Nymphomyia	Genus	6	SC
Psychodidae	**Family**	10	CG
Pericoma	Genus	4	CG
Philosepedon	Genus	10	CG
Psychoda	Genus	10	CG
Telmatoscopus	Genus	10	CG
Threticus	Genus	10	CG
Ptychopteridae	**Family**	8	CG
Bittacomorpha	Genus	8	CG
Bittacomorphella	Genus	8	CG
Ptychoptera	Genus	8	CG
Tanyderidae	**Family**	6	CG
Protoplasa	Genus	6	CG
Thaumalea	Genus	6	SC
Trichothaumalea	Genus	6	SC
Athericidae	**Family**	2	PR
Atherix	Genus	2	PR
Pelecorhynchidae	**Family**	5	PR
Glutops	Genus	5	PR
Dolichopodidae	**Family**	4	PR
Argyra	Genus	4	PR
Asyndetus	Genus	4	PR
Campsicnemus	Genus	4	CG
Dolichopus	Genus	4	PR
Hercostomus	Genus	4	PR
Hydrophorus	Genus	4	PR
Hypocharassus	Genus	4	PR
Liancalus	Genus	4	PR
Pelastoneurus	Genus	4	PR
Sympycnus	Genus	4	PR
Tachytrechus	Genus	4	PR
Telmaturgus	Genus	4	PR
Thinophilus	Genus	4	PR
Empididae	**Family**	6	PR
Chelifera	Genus	6	PR
Chelipoda	Genus	6	PR

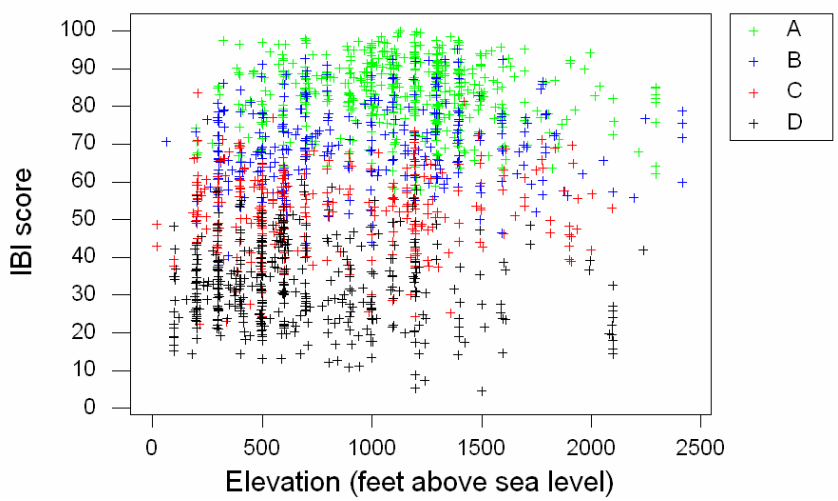
Clinocera	Genus	6	PR
Dolichocephala	Genus	5	PR
Hemerodromia	Genus	6	PR
Metachela	Genus	6	PR
Neoplasta	Genus	6	PR
Oreothalia	Genus	6	PR
Proclinopyga	Genus	6	PR
Rhamphomyia	Genus	6	PR
Roederiodes	Genus	6	PR
Stilpon	Genus	6	PR
Trichoclinocera	Genus	6	PR
Oreogeton	Genus	6	PR
Stratiomyidae	**Family**	8	CG
Caloparyphus	Genus	8	CG
Euparyphus	Genus	8	CG
Hedriodiscus	Genus	8	SC
Labostigmina	Genus	8	CG
Nemotelus	Genus	8	CG
Odontomyia	Genus	8	CG
Oxycera	Genus	8	SC
Sargus	Genus	8	CG
Stratiomys	Genus	5	CG
Tabanidae	**Family**	6	PI
Atylotus	Genus	6	PI
Chrysops	Genus	7	PI
Haematopota	Genus	6	PR
Hybomitra	Genus	6	PR
Merycomyia	Genus	6	PR
Tabanus	Genus	5	PR
Diachlorus	Genus	6	PR
Ephydridae	**Family**	6	PI
Leptopsilopa	Genus	6	CG
Psilopa	Genus	6	CG
Rhysophora	Genus	6	SH
Muscidae	**Family**	6	PR
Caricea	Genus	6	PR
Limnophora	Genus	6	PR
Lispe	Genus	6	PR
Lispoides	Genus	6	PR
Phaonia	Genus	6	PR
Spilogona	Genus	6	PR
Phoridae	**Family**	6	CG
Dohrniphora	Genus	6	CG
Megaselia	Genus	6	CG
Scathophagidae	**Family**	6	SH
Acanthocnema	Genus	6	SH
Cordilura	Genus	6	SH
Hydromyza	Genus	6	SH
Orthacheta	Genus	6	PR
Spaziphora	Genus	6	SC
Syrphidae	**Family**	10	CG
Blera	Genus	10	CG
Callicera	Genus	10	CG
Ceriana	Genus	10	CG
Chalcosyrphus	Genus	10	CG

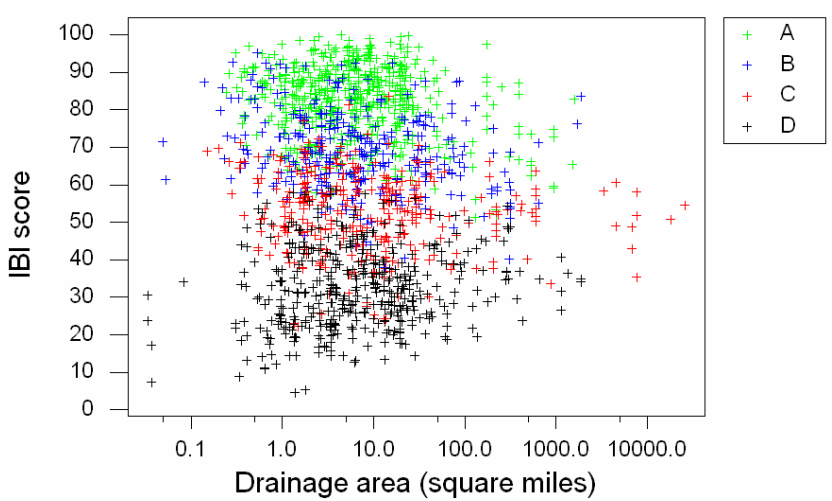
Chrysogaster	Genus	10	CG
Eristalinus	Genus	10	CG
Helophilus	Genus	10	CG
Mallota	Genus	10	CG
Myolepta	Genus	10	CG
Neoascia	Genus	10	CG
Sericomyia	Genus	10	CG
Spilomyia	Genus	10	CG
Tipulidae	**Family**	4	SH
Brachypremna	Genus	4	SH
Leptotarsus	Genus	4	SH
Prionocera	Genus	4	SH
Tipula	Genus	4	SH
Phalacrocera	Genus	4	SH
Triogma	Genus	4	SH
Antocha	Genus	3	CG
Arctoconopa	Genus	4	SH
Cryptolabis	Genus	4	CG
Dactylolabis	Genus	4	SH
Dicranota	Genus	3	PR
Elliptera	Genus	4	SH
Gonomyia	Genus	4	SH
Helius	Genus	4	SH
Hexatoma	Genus	2	PR
Limnophila	Genus	3	PR
Limonia	Genus	6	SH
Molophilus	Genus	4	SH
Ormosia	Genus	6	CG
Paradelphomyia	Genus	4	SH
Pedicia	Genus	6	PR
Pilaria	Genus	7	PR
Pseudolimnolophila	Genus	2	PR
Rhabdomastix	Genus	4	SH
Ulomorpha	Genus	4	PR
Erioptera	Genus	7	CG
Lipsothrix	Genus	4	SH
Culicidae	**Family**	8	FC
Aedes	Genus	8	FC
Anopheles	Genus	8	FC
Culex	Genus	8	FC
Culiseta	Genus	8	FC
Mansonia	Genus	8	FC
Orthopodomyia	Genus	8	FC
Psorophora	Genus	8	PR
Toxorhynchites	Genus		PR
Uranotaenia	Genus	8	FC
Wyeomyia	Genus	8	FC
Simuliidae	**Family**	6	FC
Cnephia	Genus	4	FC
Ectemnia	Genus	1	FC
Greniera	Genus	6	FC
Prosimulium	Genus	2	FC
Simulium	Genus	6	FC
Stegopterna	Genus	6	FC
Twinnia	Genus	6	FC

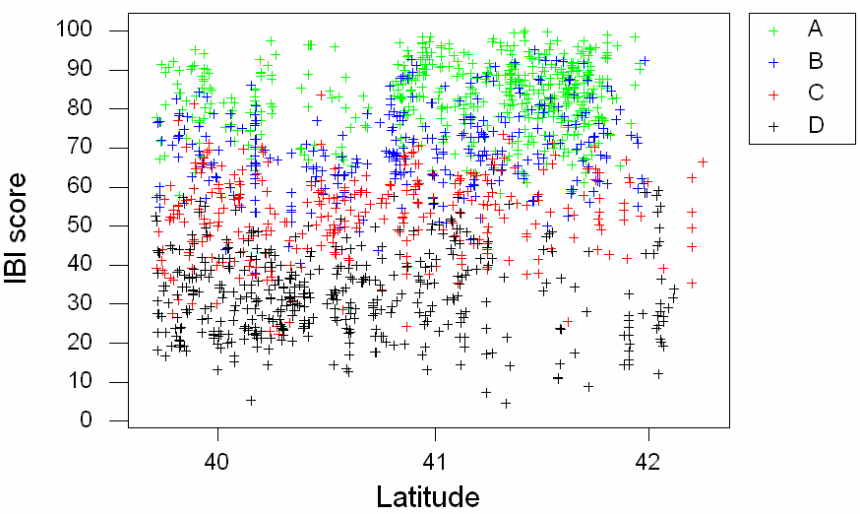
Chironomidae	**Family**	6	CG
Sciomyzidae	**Family**	10	PR
Demosongea	**Class**		
Haploscleriana	**Order**		
Spongillidae	**Family**	4	FC
Hydrozoa	**Class**		
Hydroida	**Order**		
Hydridae	**Family**	4	PR
Cavidae	**Family**	4	PR
Trachylina	**Order**		
Petasidae	**Family**	4	PR
Turbellaria	**Class**	9	PR
Nemertea	***Generic***	6	PR
Nematoda	***Generic***	9	CG
Gastropoda	**Class**		
Mesogastropoda	**Order**		
Valvatidae	**Family**	2	SC
Viviparidae	**Family**	7	CG
Ampullaridae	**Family**	7	SC
Bithyniidae	**Family**	7	SC
Micromelaniidae	**Family**	7	SC
Hydrobiidae	**Family**	8	SC
Pomatiopsidae	**Family**	8	SC
Pleuroceridae	**Family**	7	SC
Lymnaeidae	**Family**	7	SC
Physidae	**Family**	8	SC
Planorbidae	**Family**	6	SC
Ancylidae	**Family**	7	SC
Bivalvia	**Class**		
Unionoida	**Order**		
Margaritiferidae	**Family**	5	FC
Unionidae	**Family**	4	FC
Sphaeriidae	**Family**	8	FC
Pisidium	Genus	8	FC
Musculium	Genus	8	FC
Sphaerium	Genus	8	FC

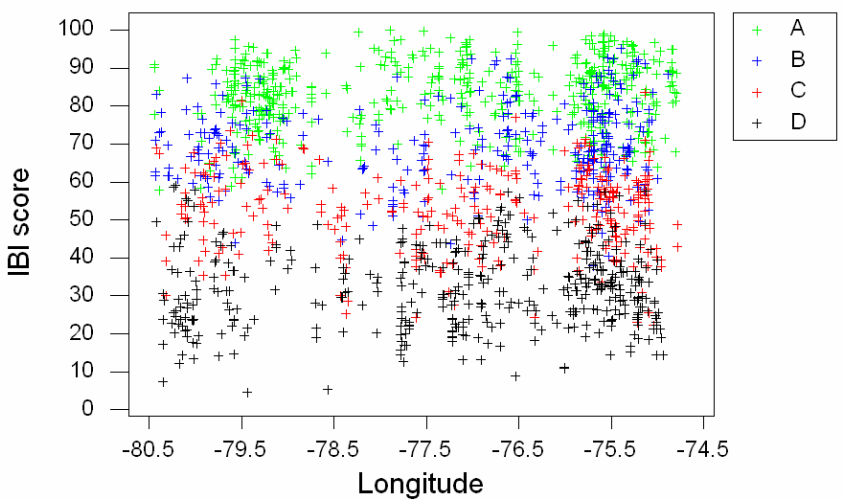
Corbiculidae	**Family**	4	FC
Dreissenidae	**Family**	5	FC
Hirudinea	**Class**	8	PR
Oligochaeta	**Class**	10	CG
Tubificidae	**Family**	10	CG
Branchiobdellida	**Order**	6	CG
Polychaeta	**Class**	10	FC
Crustacea	**Class**		
Amphipoda	**Order**	6	CG
Crangonyctidae	**Family**	4	CG
Crangonyx	Genus	4	CG
Stygonectes	Genus	4	CG
Gammaridae	**Family**	4	CG
Gammarus	Genus	4	CG
Haustoriidae	**Family**	5	CG
Monoporeia	Genus	5	CG
Pontoporeiidae	**Family**	5	CG
Talitridae	**Family**	8	CG
Hyalella	Genus	8	CG
Decapoda	**Order**		UK
Cambaridae	**Family**	6	CG
Cambarus	Genus	6	CG
Fallicambarus	Genus	6	CG
Orconectes	Genus	6	CG
Procambarus	Genus	6	SH
Isopoda	**Order**	8	CG
Asellidae	**Family**	8	CG
Caecidotea	Genus	6	CG
Lirceus	Genus	8	CG
Cladocera	**Order**	5	FC
Ostracoda	**Phylum**	8	CG
Bryozoa	**Phylum**	4	FC
Hydracarina	***Generic***	7	PR
Nematomorpha	**Phylum**	9	CG

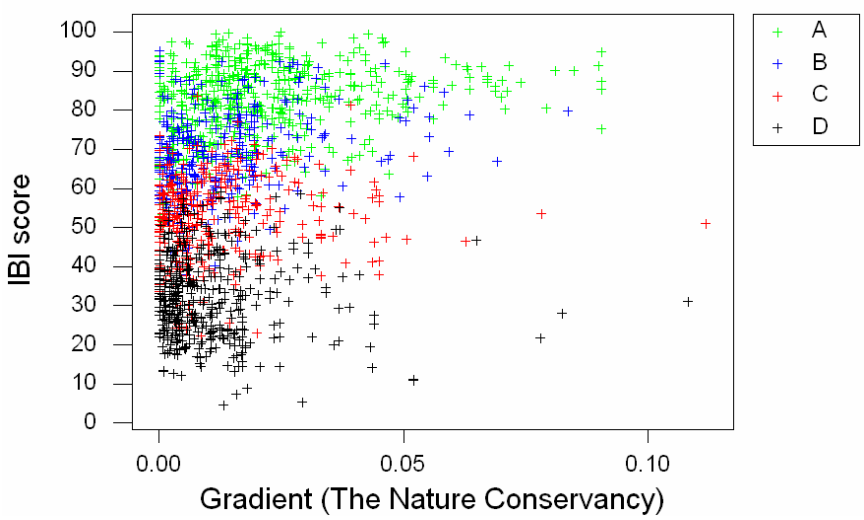
Geographic


IBI score
Elevation (feet above sea level)
A
B
C
D

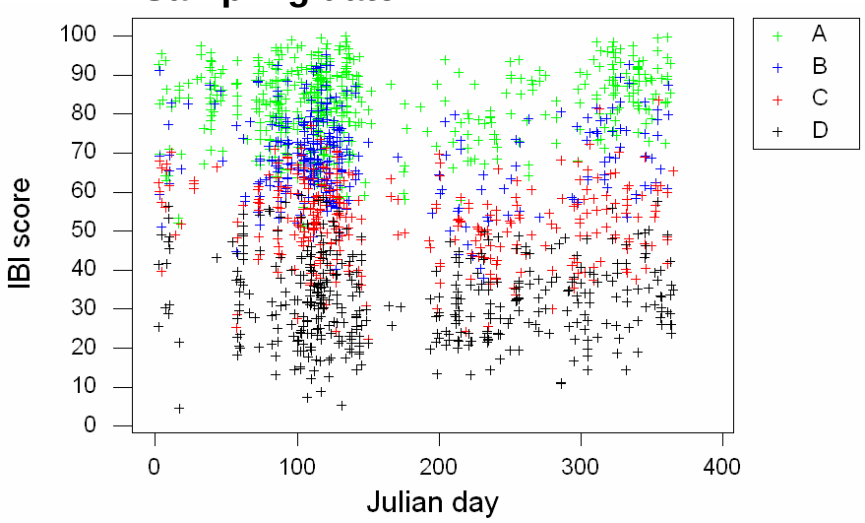

IBI score
Drainage area (square miles)
A
B
C
D


IBI score
Latitude
A
B
C
D

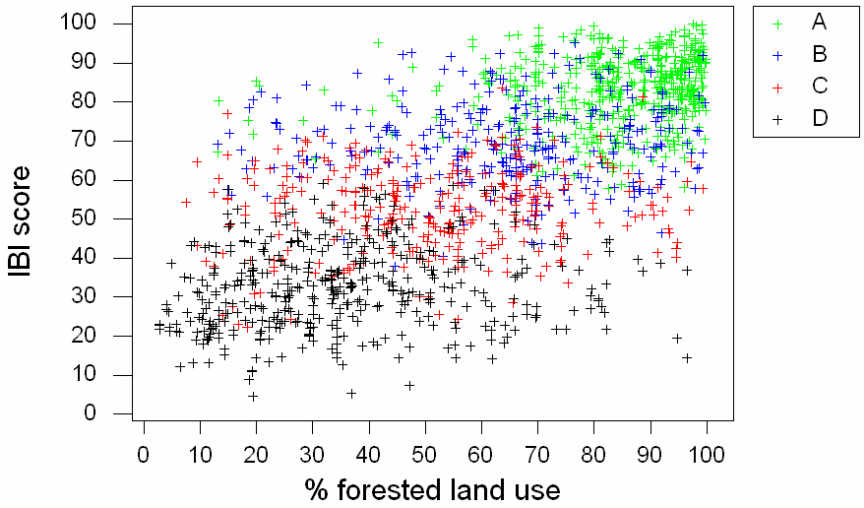

IBI score
Longitude
A
B
C
D

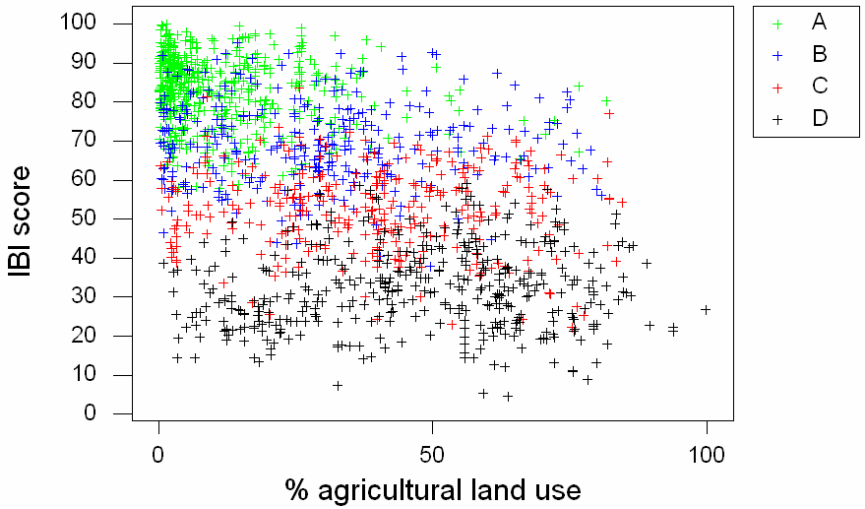


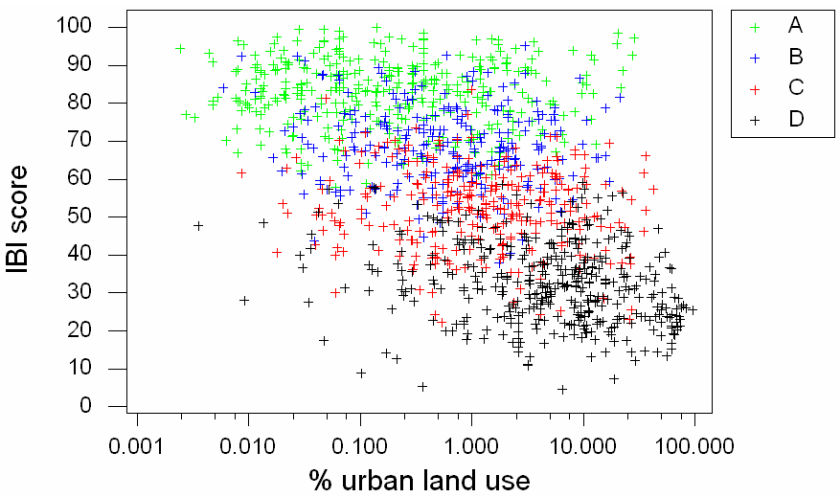
Sampling date



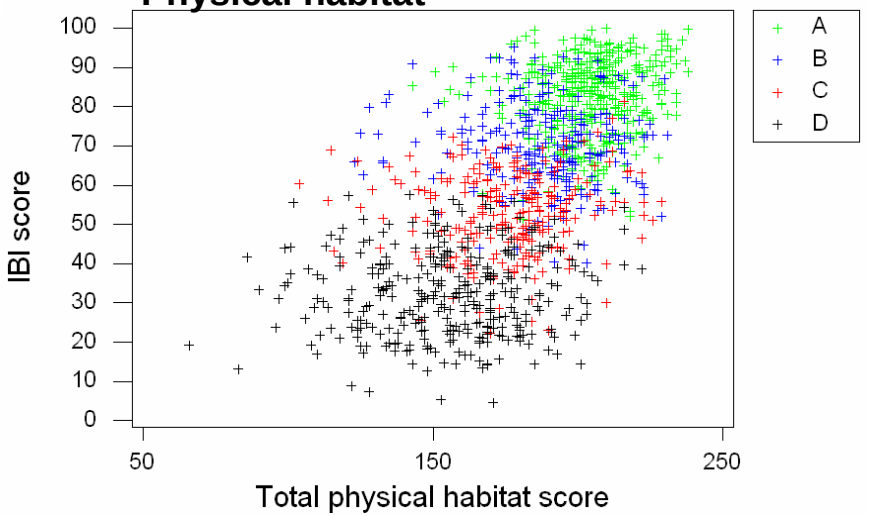
Land use



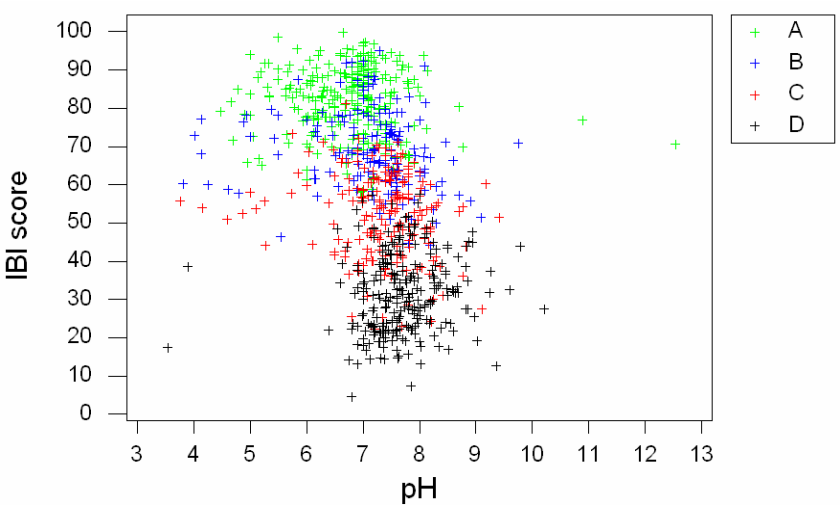


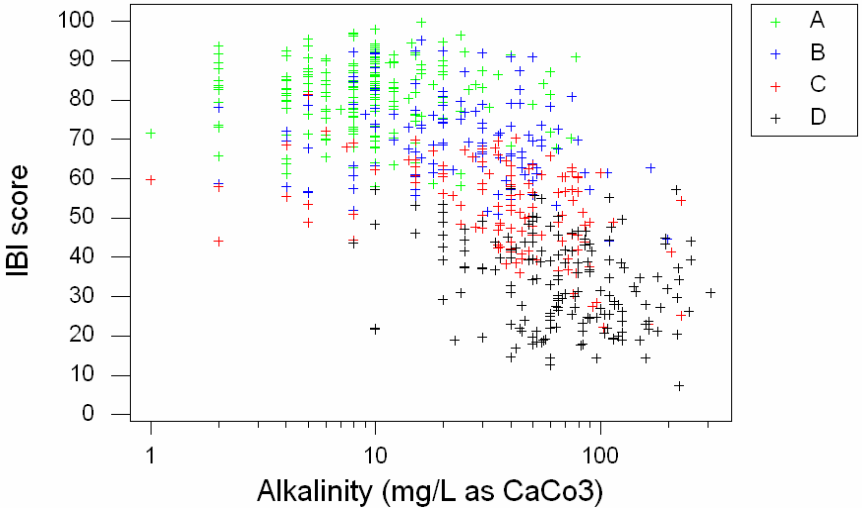


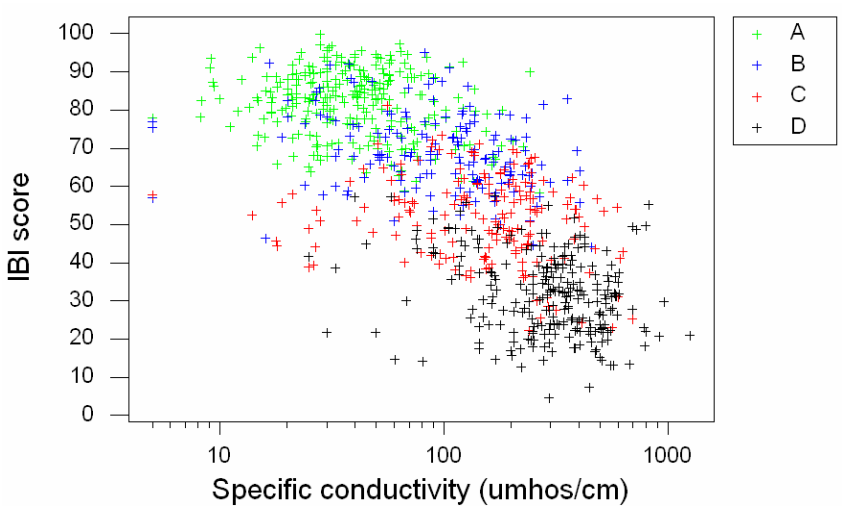
Physical habitat

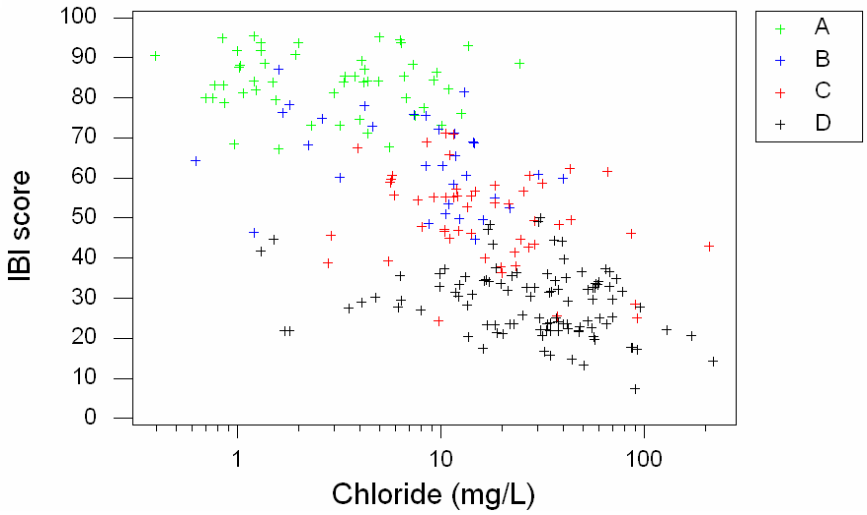


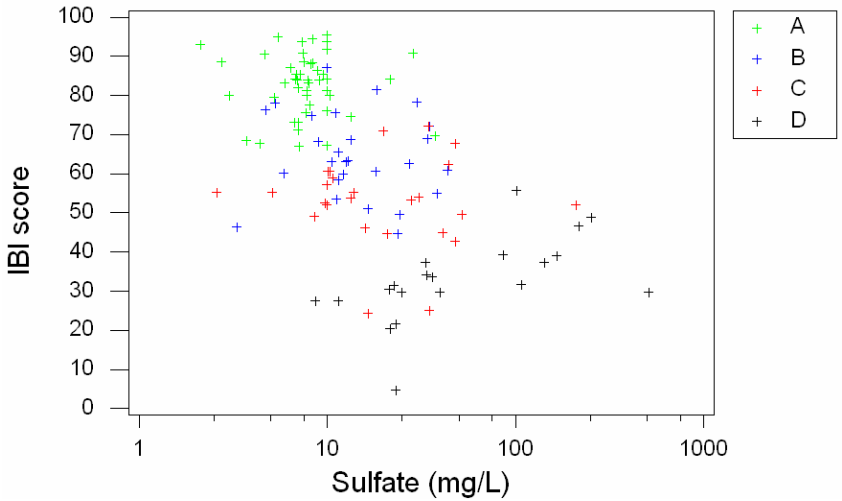
Water chemistry

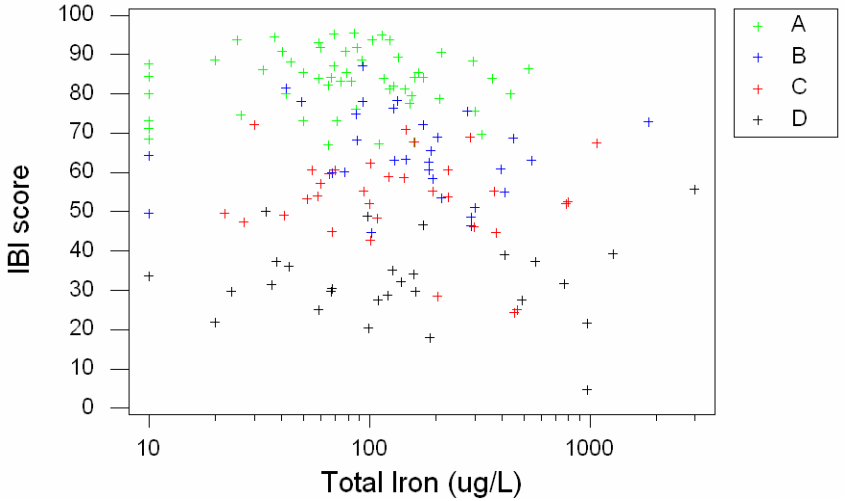


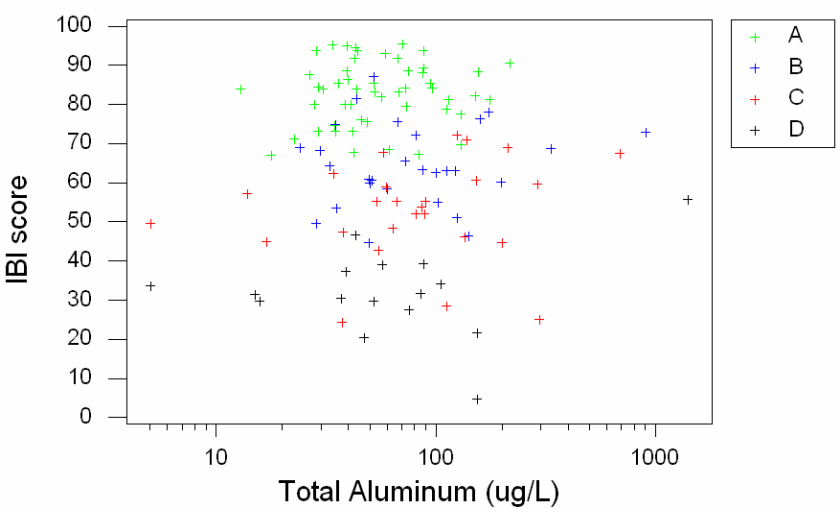


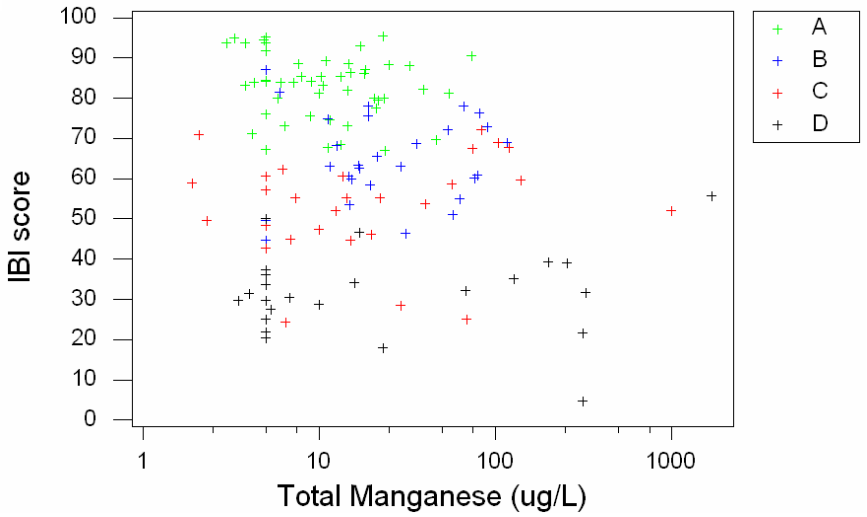

IBI score
Specific conductivity (umhos/cm)
A
B
C
D

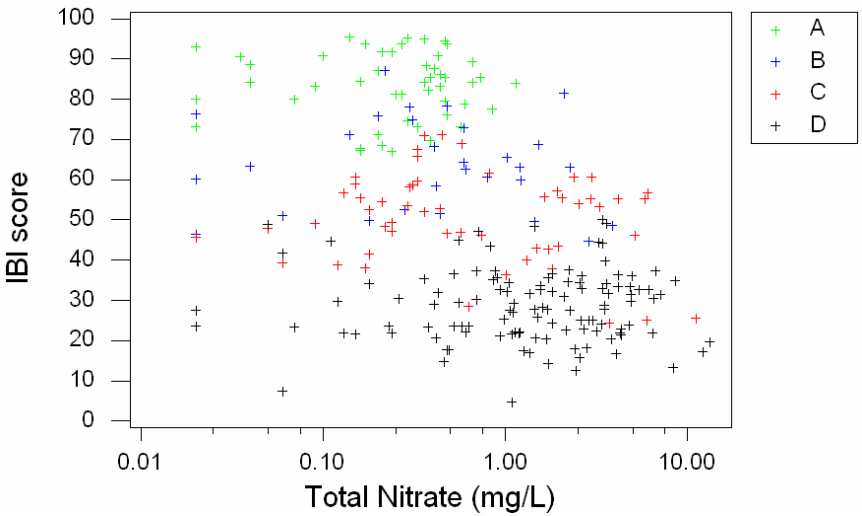

IBI score
Chloride (mg/L)
A
B
C
D

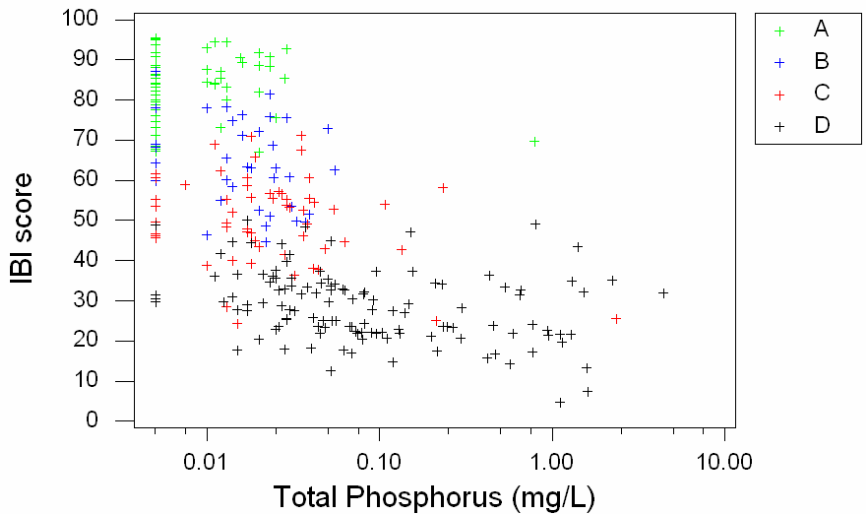

IBI score
Sulfate (mg/L)
A
B
C
D


IBI score
Total Iron (ug/L)
A
B
C
D


IBI score
100
90
80
70
60
50
40
30
20
10
0
10
100
1000
Total Aluminum (ug/L)
A
B
C
D


IBI score
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
Total Manganese (ug/L)
A
B
C
D


IBI score
100
90
80
70
60
50
40
30
20
10
0
0.01
0.10
1.00
10.00
Total Nitrate (mg/L)
A
B
C
D


IBI score
100
90
80
70
60
50
40
30
20
10
0
0.01
0.10
1.00
10.00
Total Phosphorus (mg/L)
A
B
C
D